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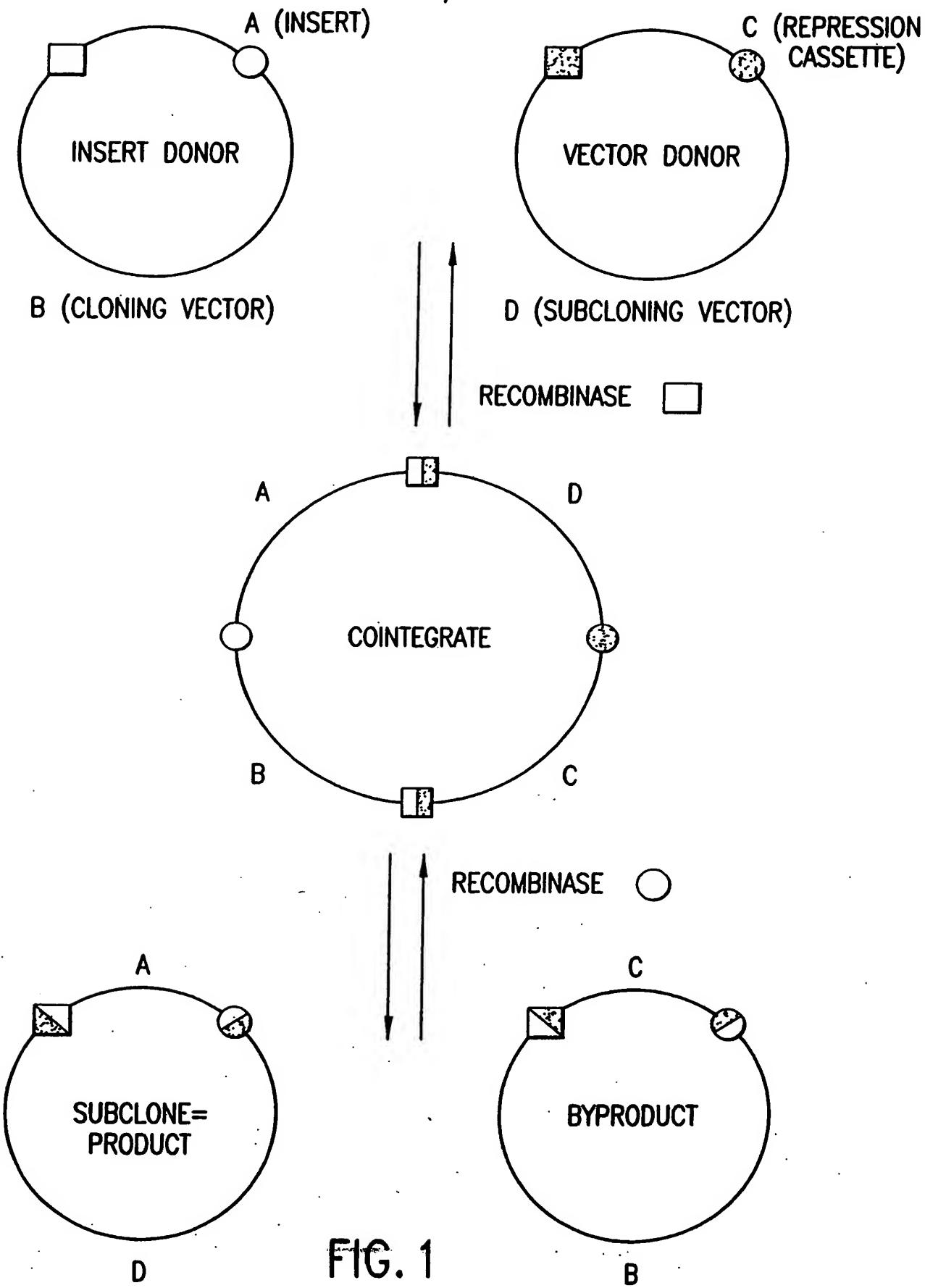


FIG. 1

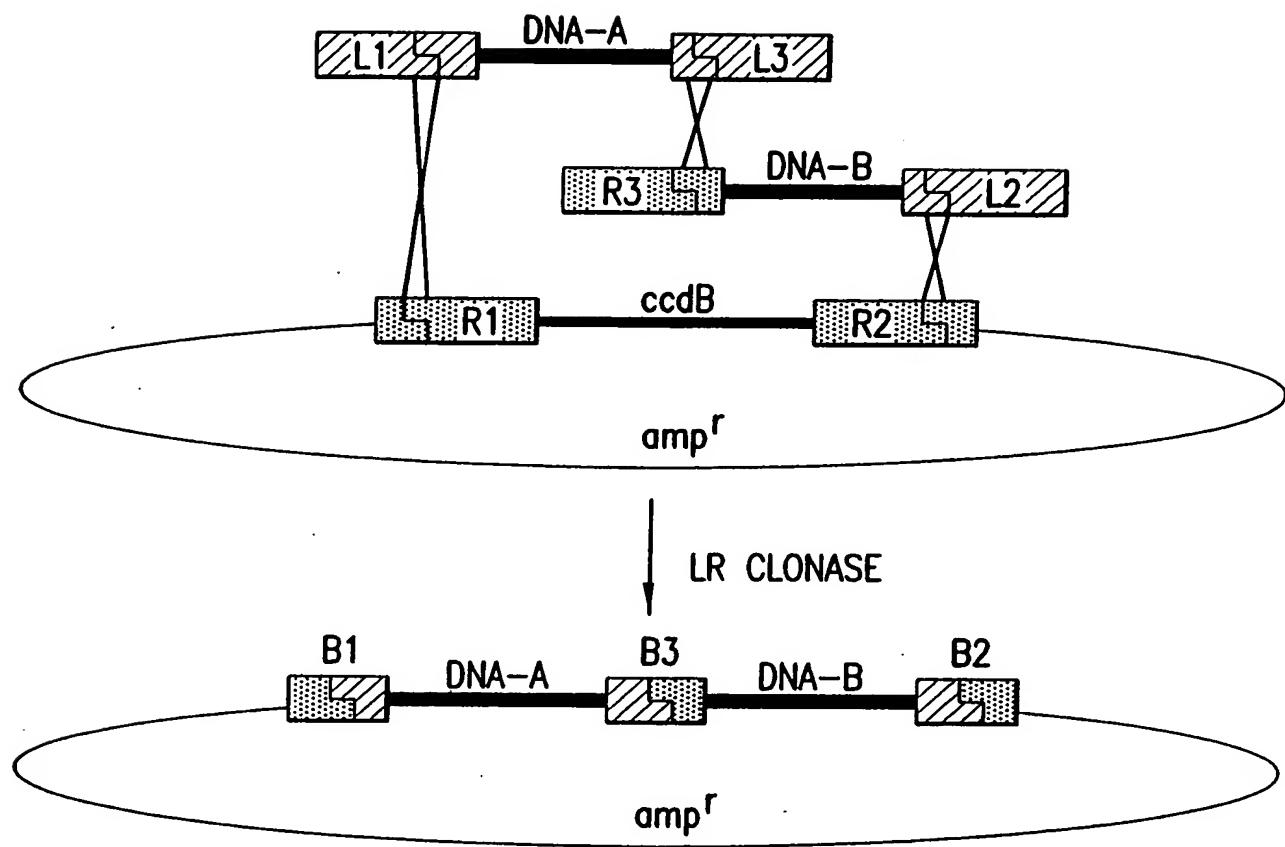


FIG. 2

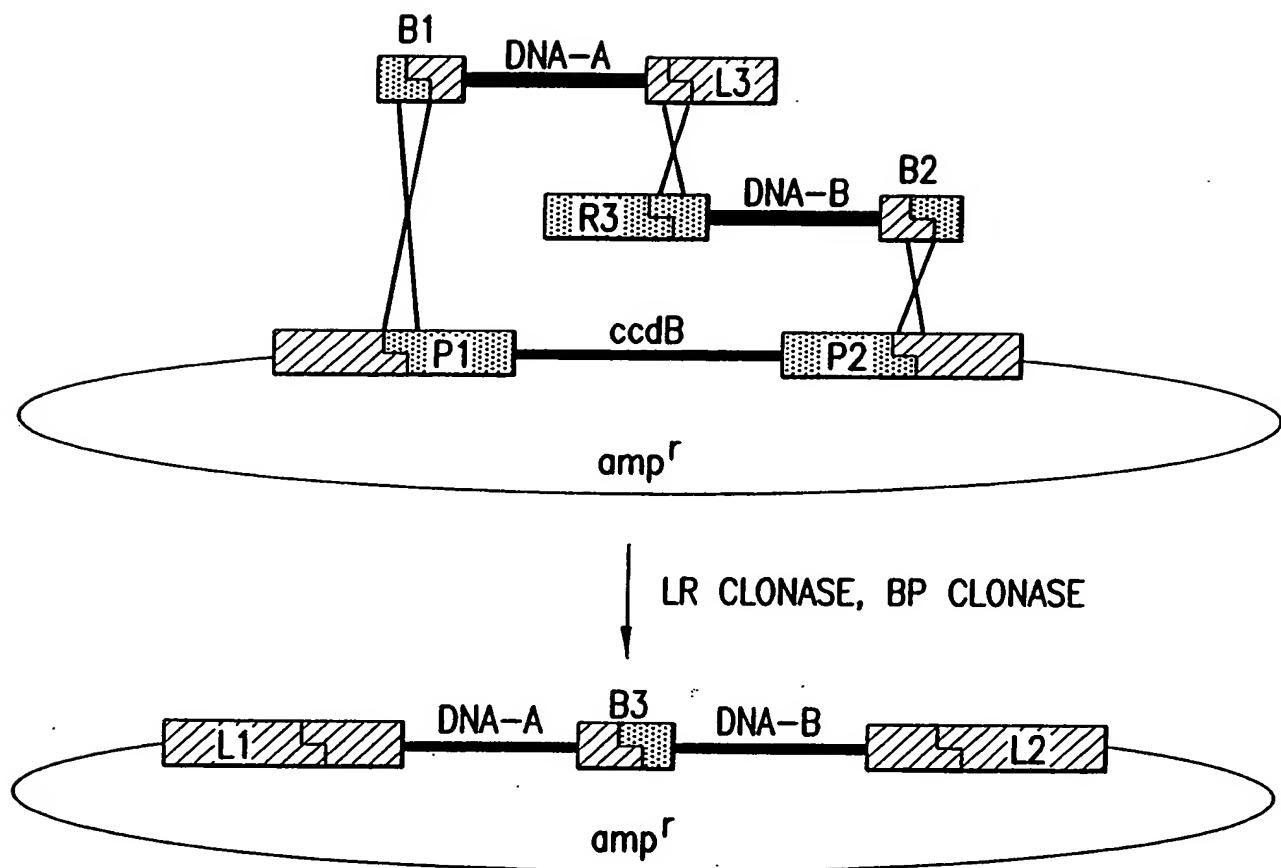


FIG. 3

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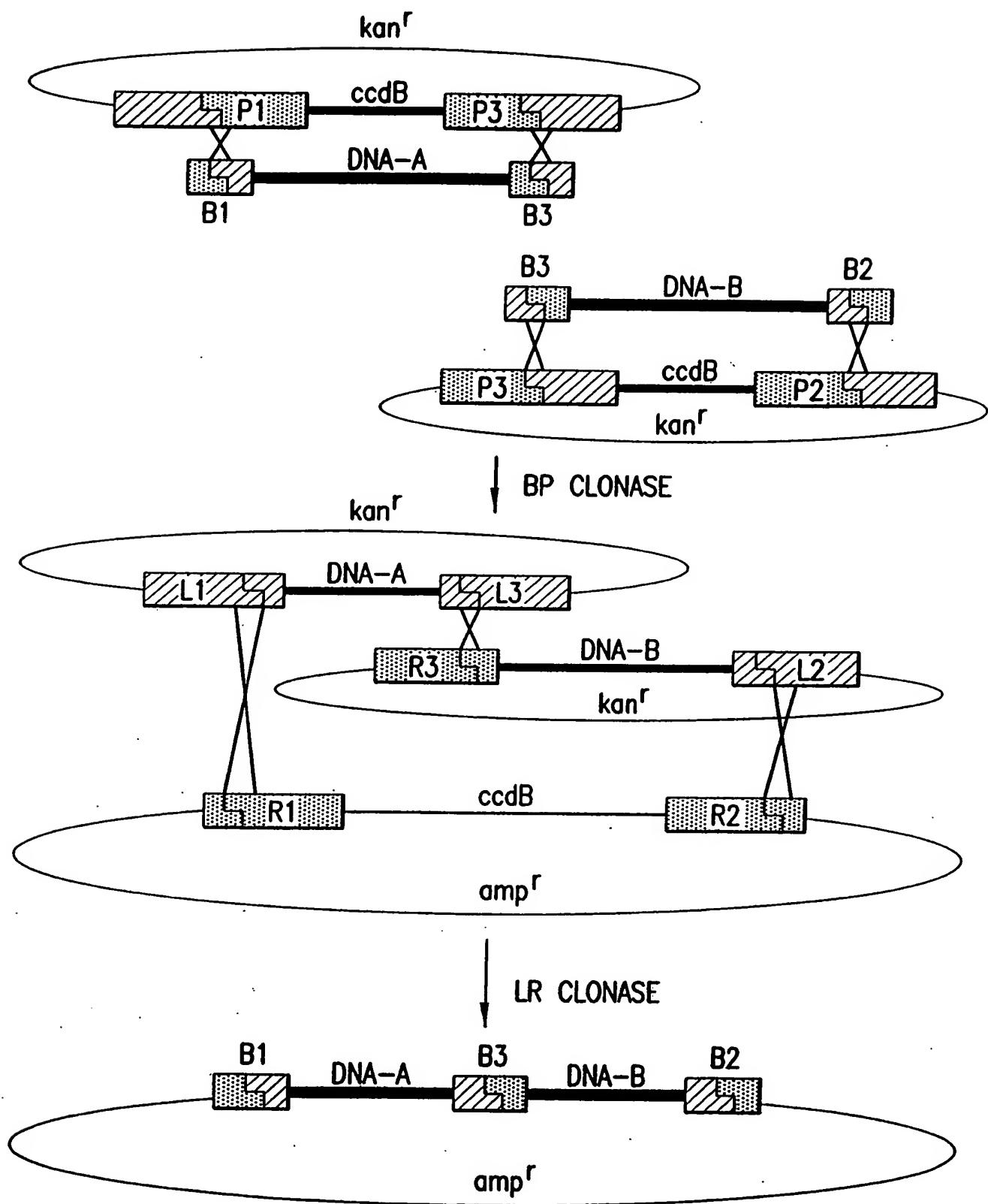
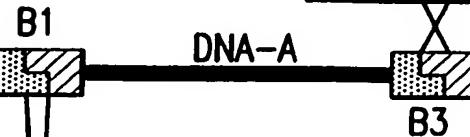
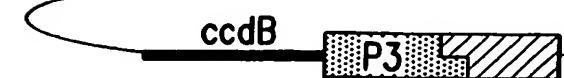


FIG. 4

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kan^r



kan^r

ccdB

P3

kan^r

P2

BP CLONASE

R3

ccdB

kan^r

DNA-A

L3

L1

DNA-B

L2

R3

ccdB

kan^r

LR CLONASE

B3

DNA-A

DNA-B

L2

kan^r

FIG. 5

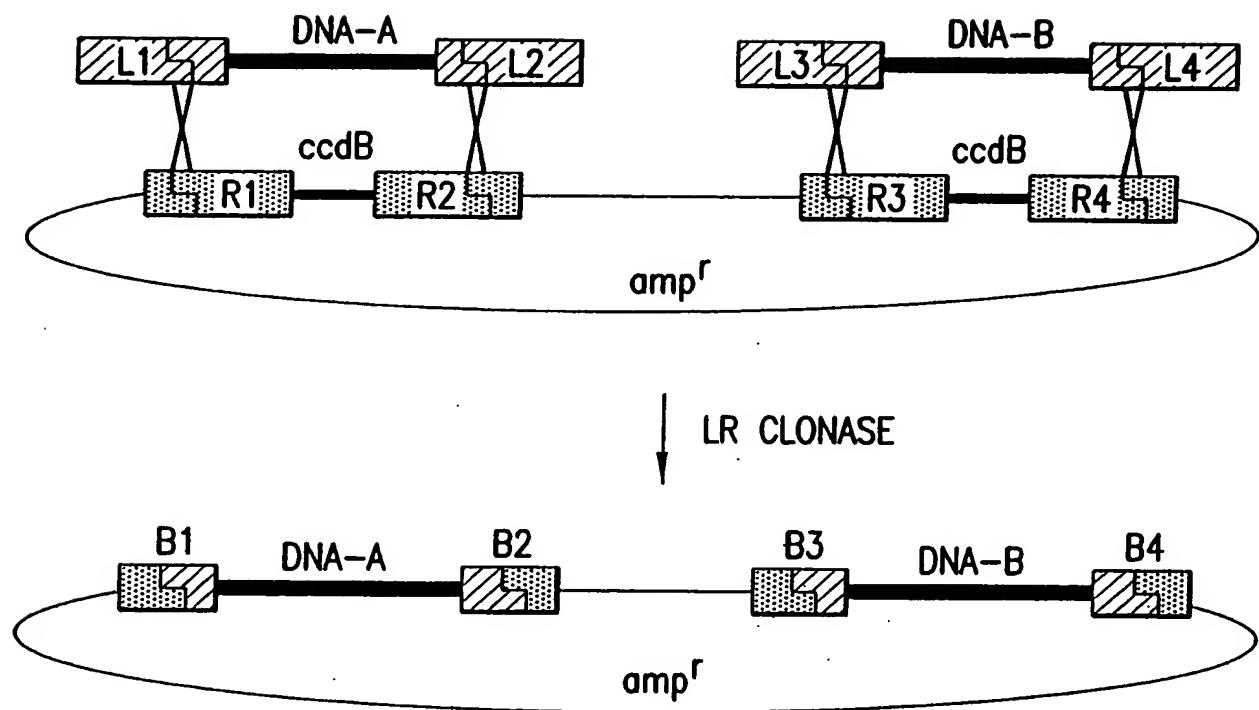


FIG. 6

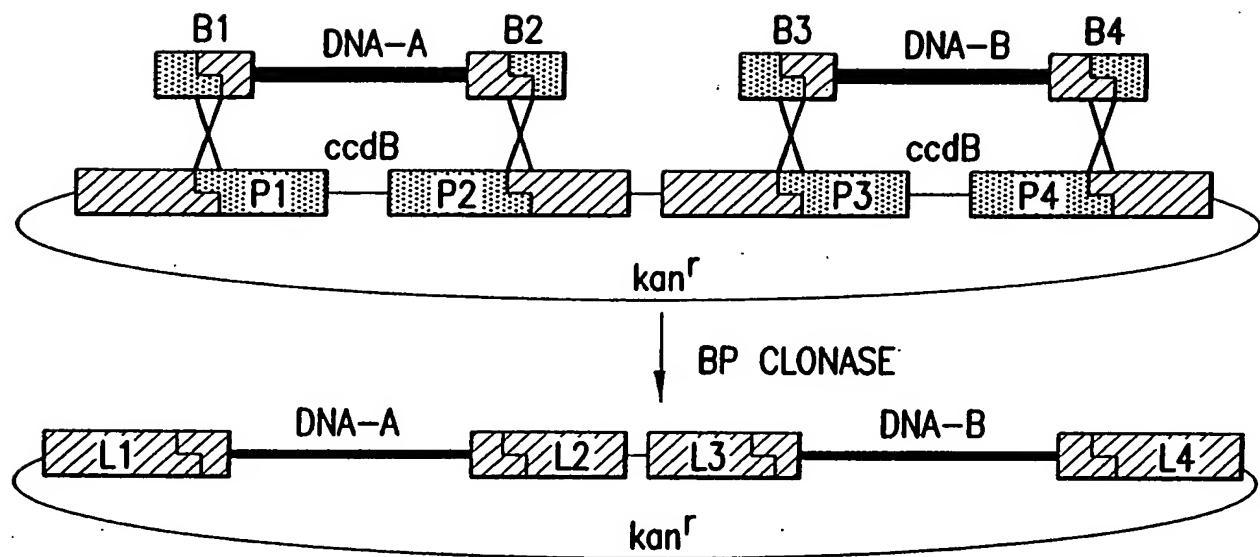


FIG. 7

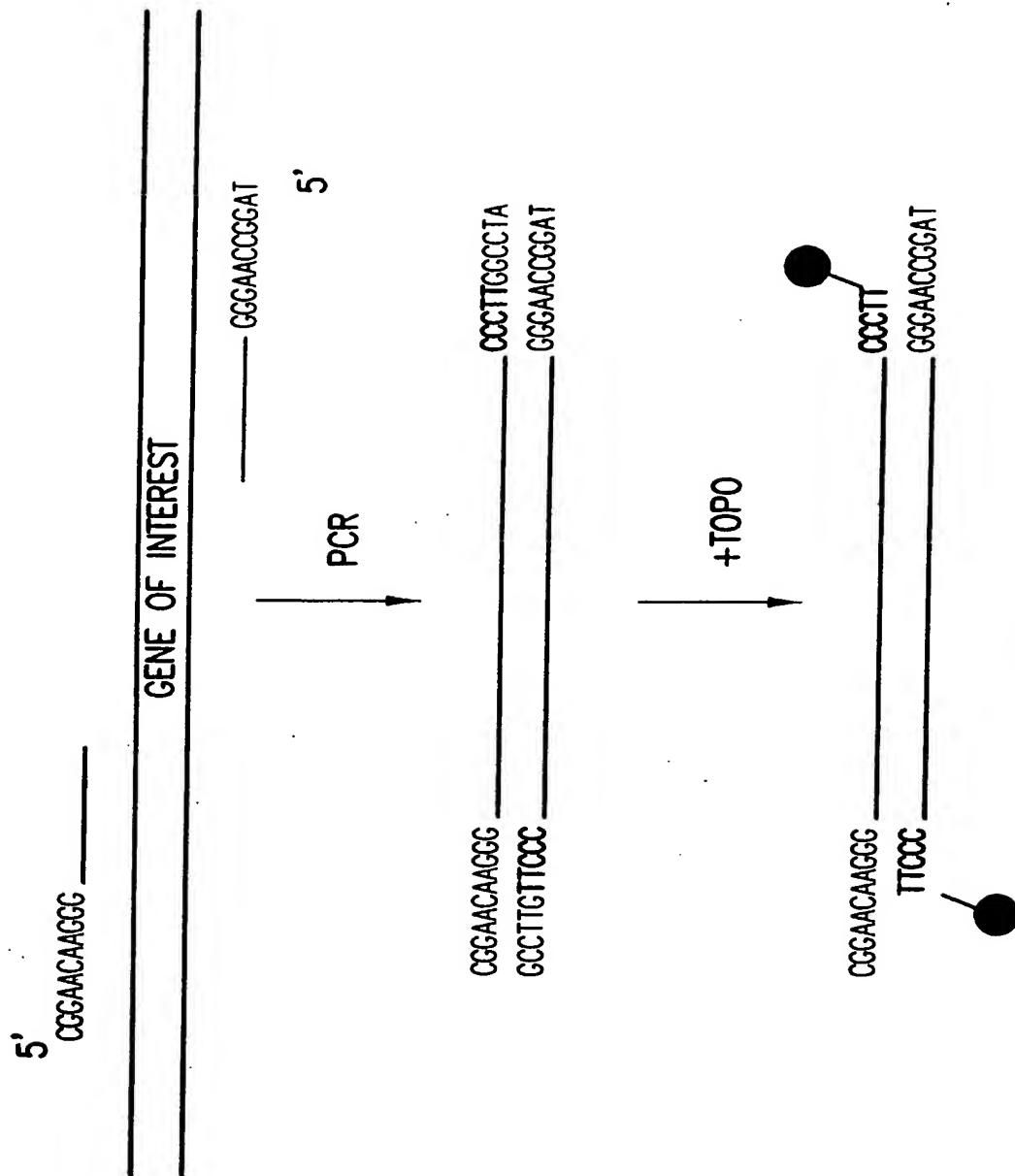


FIG. 8A

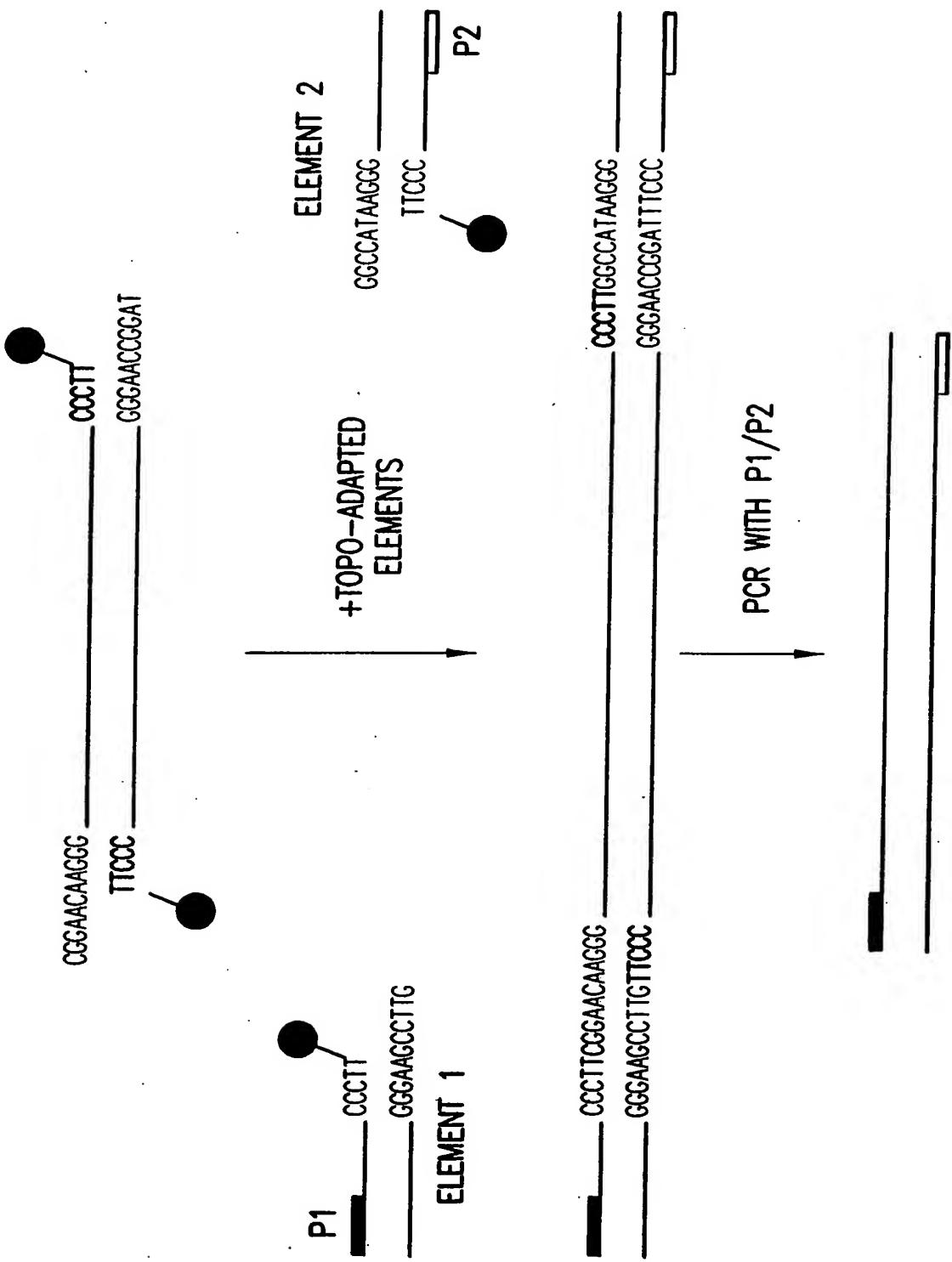


FIG. 8B

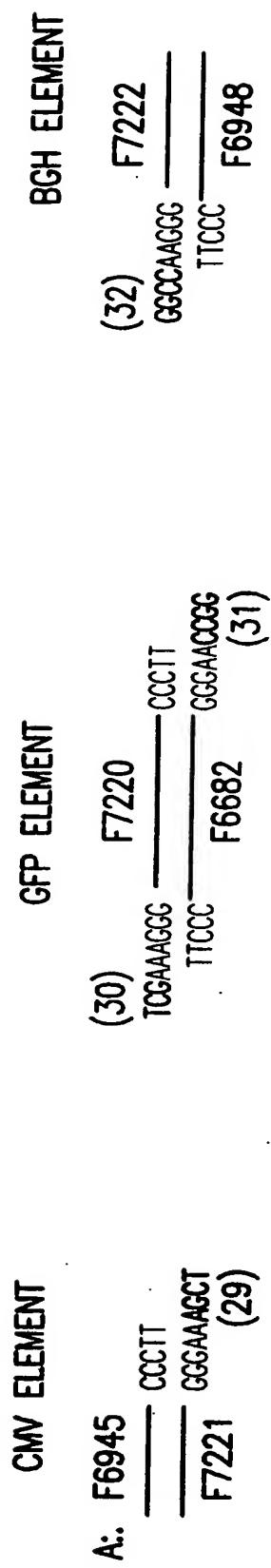
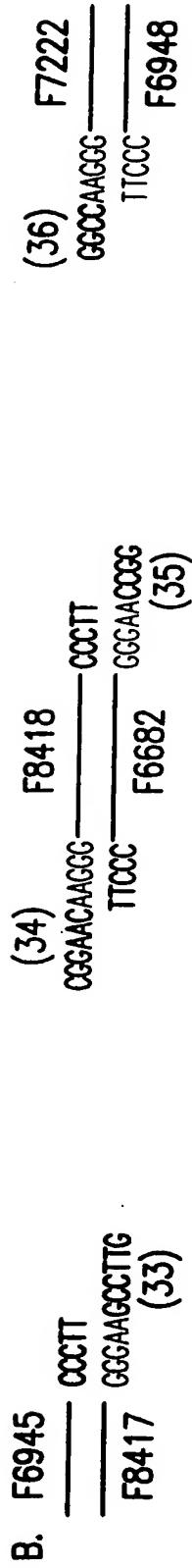
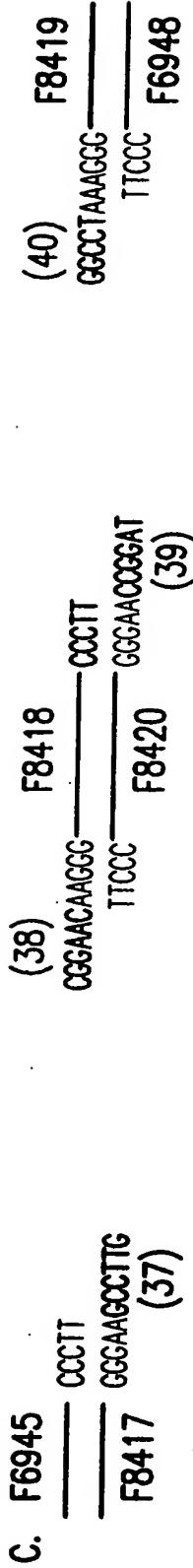
**FIG. 9A****FIG. 9B****FIG. 9C**

TABLE 1

Primer name	F#	Sequence (5'→ 3')	SEQ ID NO:
MTH1	10779	TATGTATCATACACATACGATTAGGT	1
MTH2	10780	ACCGCCTCTCCCCGGCGTT	2
CAL4r2	12667	GTTCCGAAGGGGGCAACAGTCAACTGTCTTC	3
MTH5	12505	TTGGCCAAGGGTATCTAGAAGCTCTGCAGACGGT	4
VP16r2	12668	GTTCCGAAGGGCCACCGTACTCGTCAATTCCAAG	5
SV40pAf	12016	GGCCAAAAGGGAACTTGTTATTGAGCTTATAATG	6
SV40pAr	561	CTCTGACTTGAGGTGAGATTT	7
p53f2	12669	CGGAACAAGGGGAATTCCCTGTCAACGAGACC	8
SVf2	12670	CGGAACAAGGGGAATTCCCGGGATCTGAAATTC	9
CMVr2	7221	TCGAAAGGGTCAAGGTCGACCTGCAGCTG	10
CMVf	6945	AATTCACATTGATTATTGAGTAGTTA	11
GFP-Xhof	7220	TCGAAAGGGTAATGCCAGCAAAGGAGAAAG	12
GFP-Notr	6682	GGCCAAGGGTTCTAGAGCTCATCCAT	13
BGHf2	7222	GGCCAAGGGTCTGAATGGGCCGCATAGT	14
BGHR	6948	AAGCCATAGAGCCCCGGCCA	15
CMVr3	8417	GTTCCGAAGGGTCAAGGTCGACCTGCAGCTG	16
GFPf3	8418	CGGAACAAGGGATGCCAGCAAAGGAGAAAG	17
GFP'r3	8420	TAGGCCAAGGGTTGTAGAGCTCATCCATGC	18
BGHf3	8419	GGCCTAAAGGTGAATGGGCCGCATAGT	19
T7top	9304	GAAGGAGTAATACGACTCACTATAAGGGAGCCACCATGGGCCCTTGGAAC	20
T7bottom	9305	GTTCCGAAGGGCCCATTGGGGCTCCCTATAGTGAGTGTATTACTCCTTC	21
T7amp	9306	GAAGGAGTAATACGACTCACT	22
T3top	9661	GGCCTAAAGGTCCCTTAGTGAGGGTTAATTGCGGCC	23
T3bottom	9662	GCGCGCAATTAAACCTCACTAAAGGGACCCCTTGGCC	24
lacZf2	10632	CGGAACAAGGGATGATAAGATCCCGTGTGTTTACA	25
lacZ1k2	10770	TAGGCCAAGGGGACCATTTCAATCGCACCT	26
lacZ2k2	10771	TAGGCCAAGGGGAGGCACCTCAACGCTTGGCA	27
lacZ3k2	10772	TAGGCCAAGGGTTGACACCGACCAACTGGTA	28

FIG. 9D

SAMPLE #	GAL4+PA	VP16+PA	pGene/lacZ	GAL4+p53+PA	VP16+T+PA	p53-VP16
1			0.26 μ g	0.37 μ g	0.37 μ g	
2			0.4 μ g	0.3 μ g	0.3 μ g	
3			0.4 μ g			0.6 μ g
4			0.4 μ g	10.3 μ g	10.3 μ g	
5			10.3 μ g	0.4 μ g	10.3 μ g	
6	10.3 μ g		0.4 μ g		10.3 μ g	
7			0.4 μ g	4.5 μ l PCR	4.5 μ l PCR	
8			4.5 μ l PCR	0.4 μ g	4.5 μ l PCR	
9	4.5 μ l PCR		0.4 μ g		4.5 μ l PCR	

FIG. 10A

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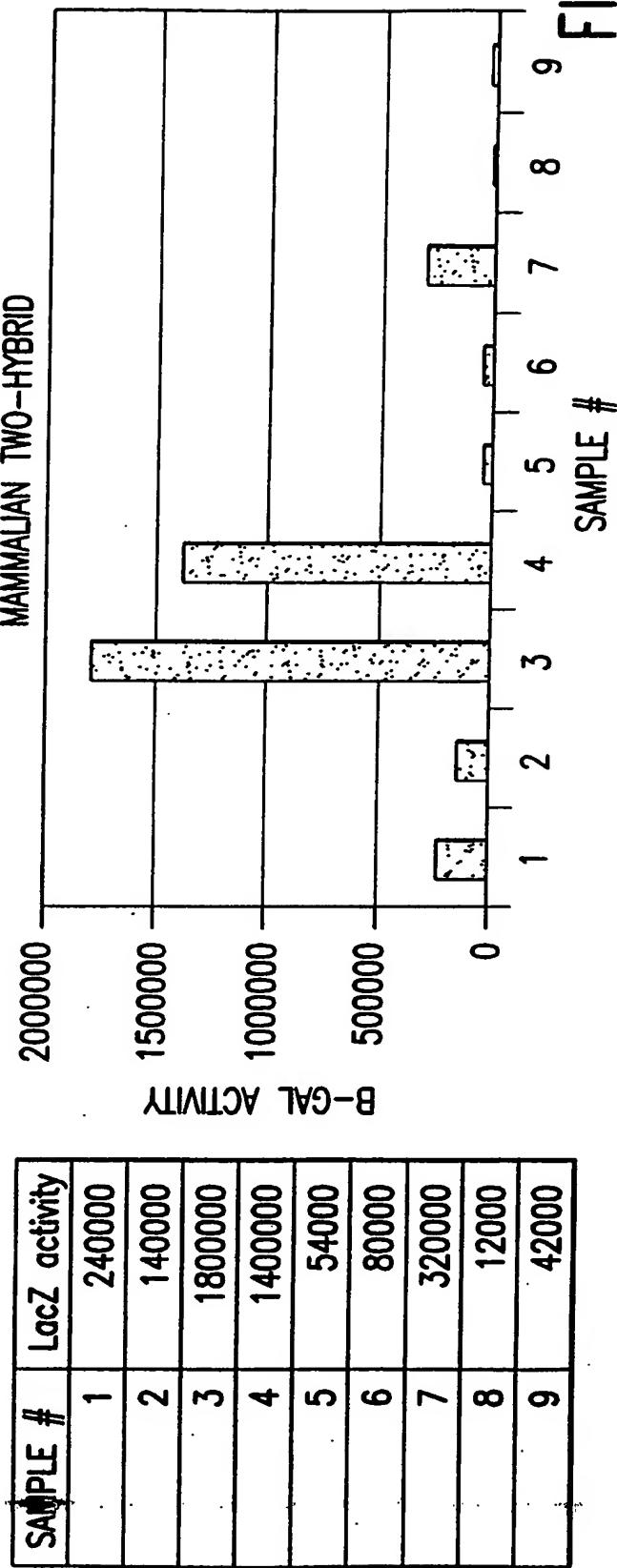


FIG. 10B
SAMPLE #

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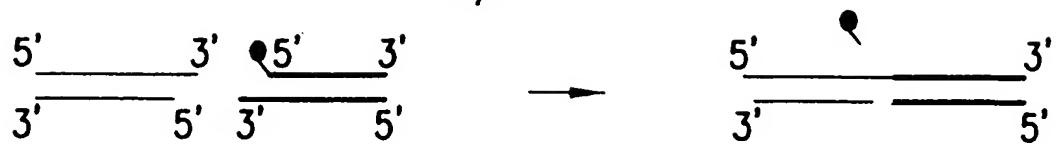


FIG. 11A

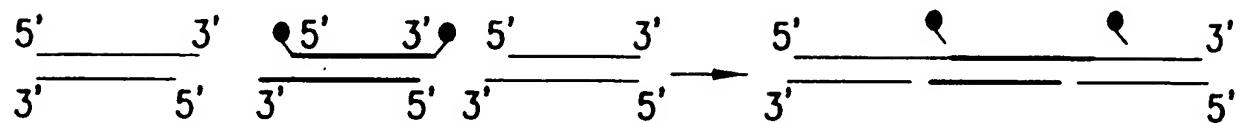


FIG. 11B

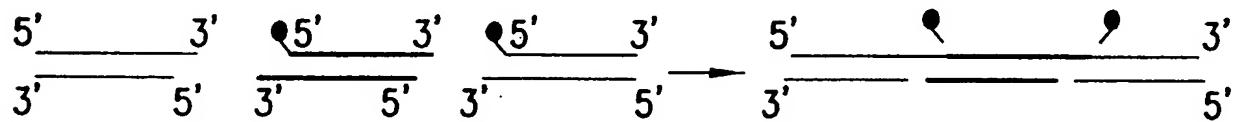


FIG. 11C

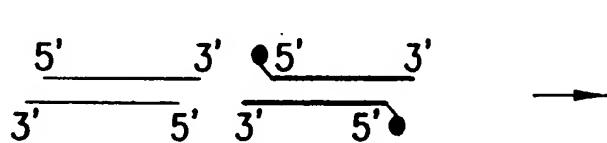


FIG. 11D

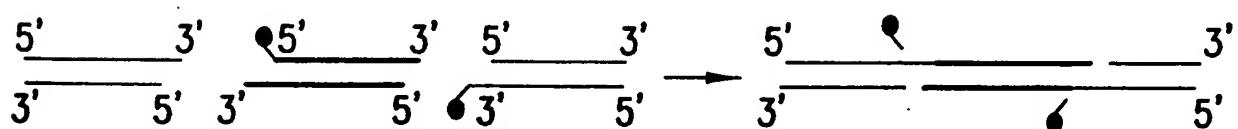


FIG. 11E

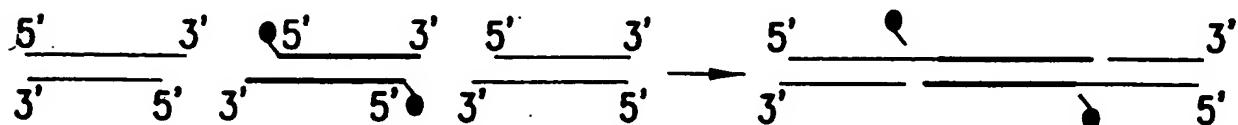


FIG. 11F

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FIG. 12A



FIG. 12B



FIG. 12C

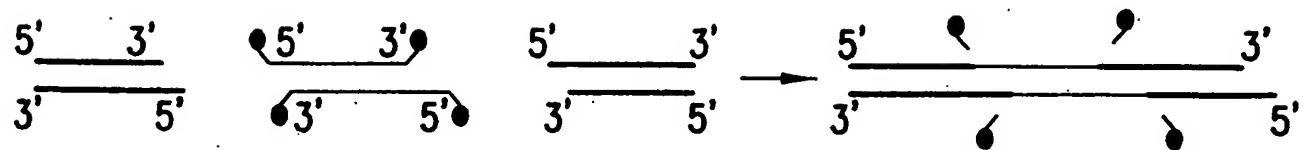


FIG. 12D

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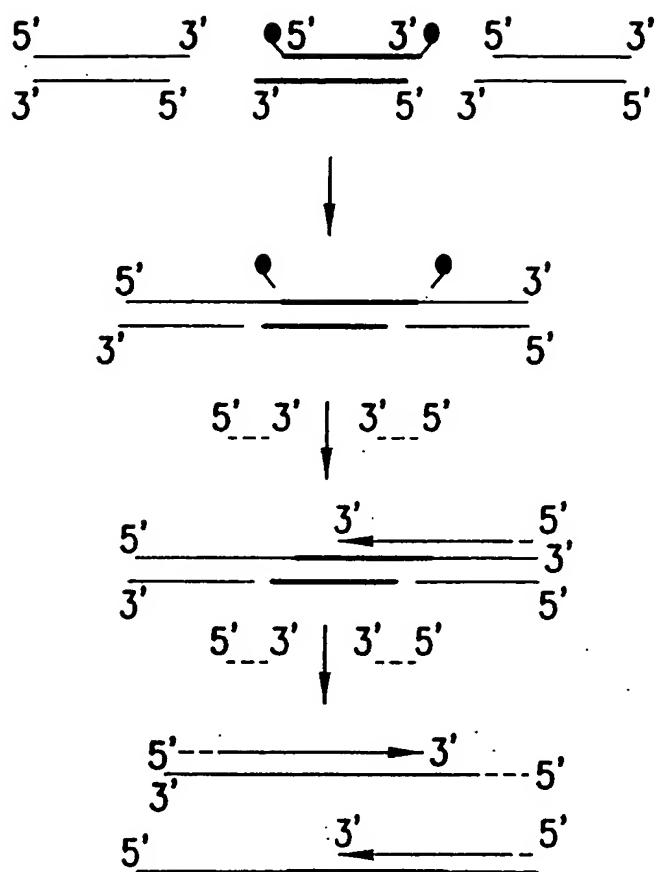


FIG. 13

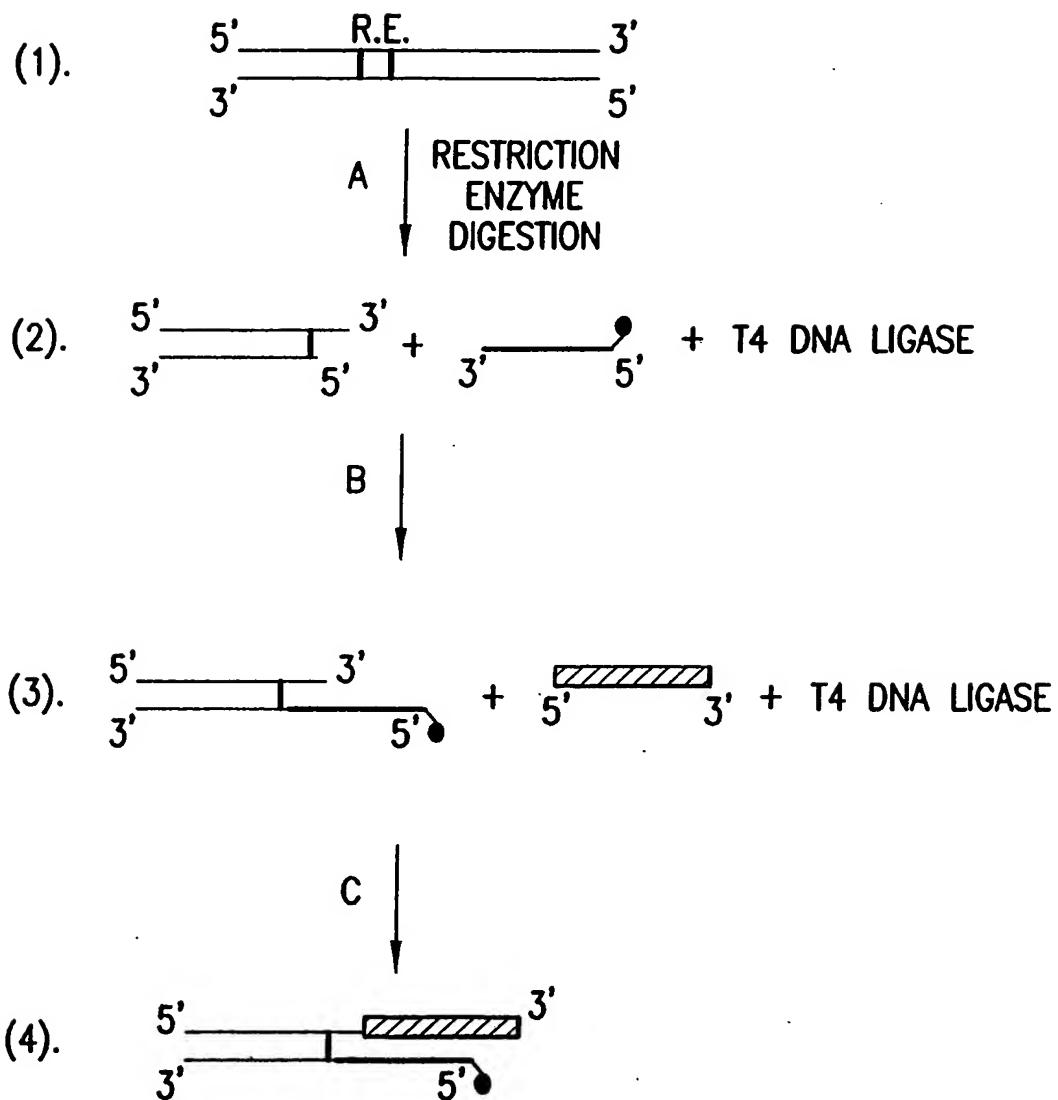


FIG. 14

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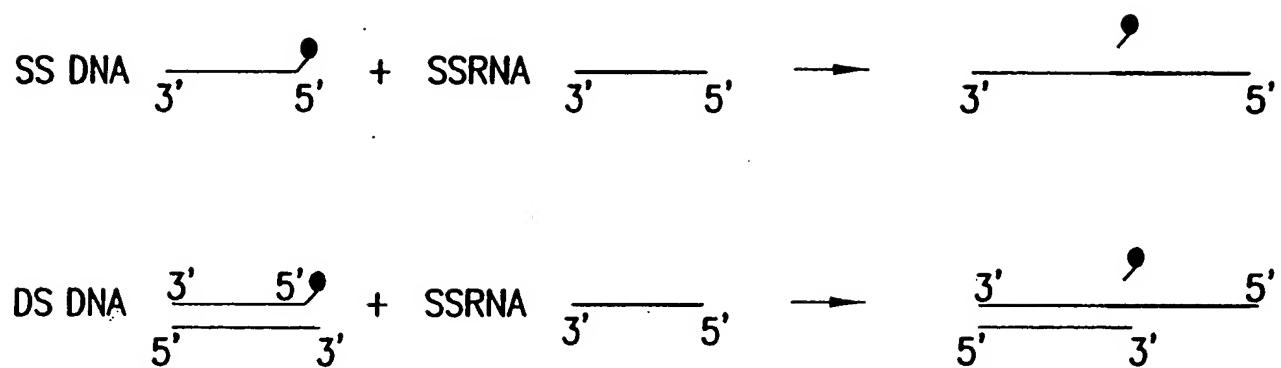


FIG. 15

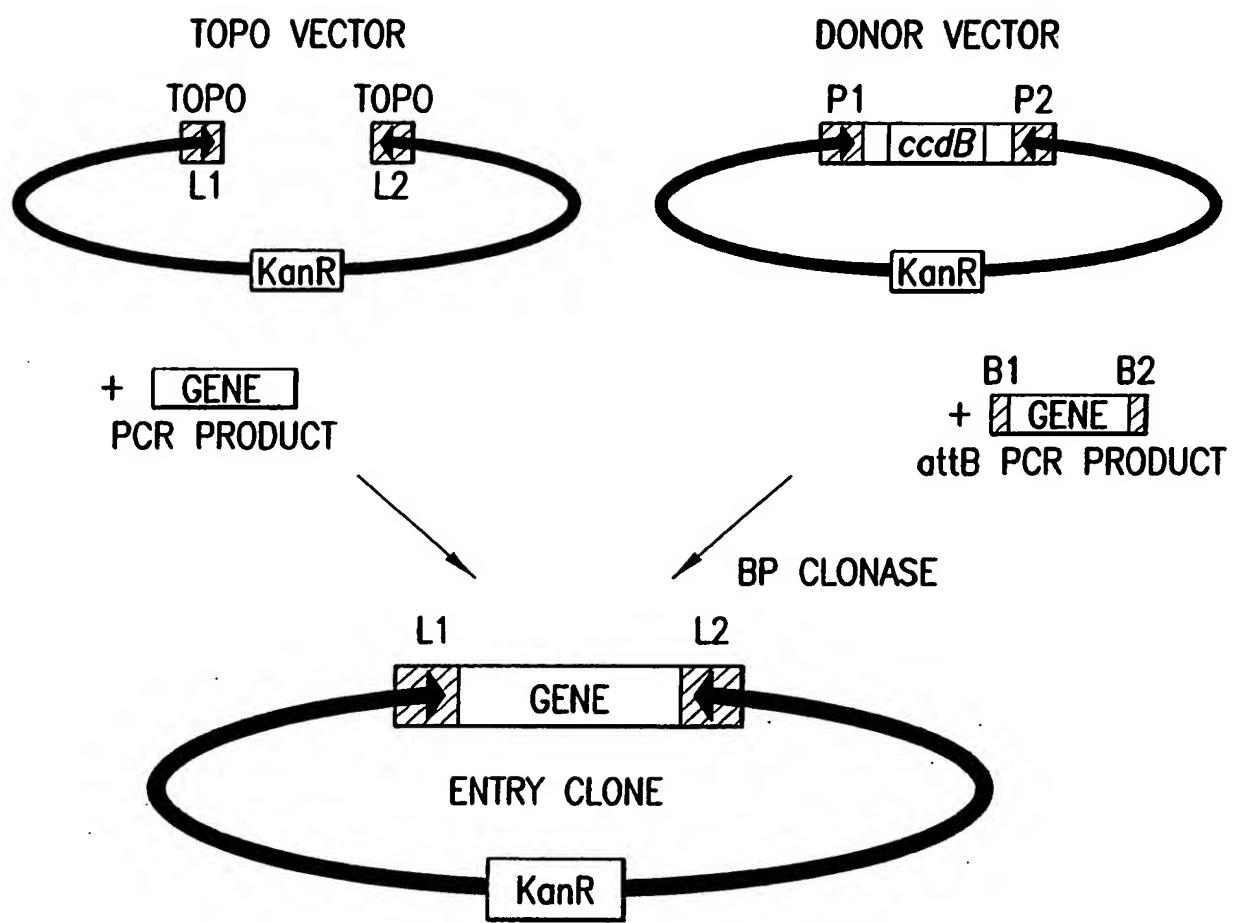


FIG. 16

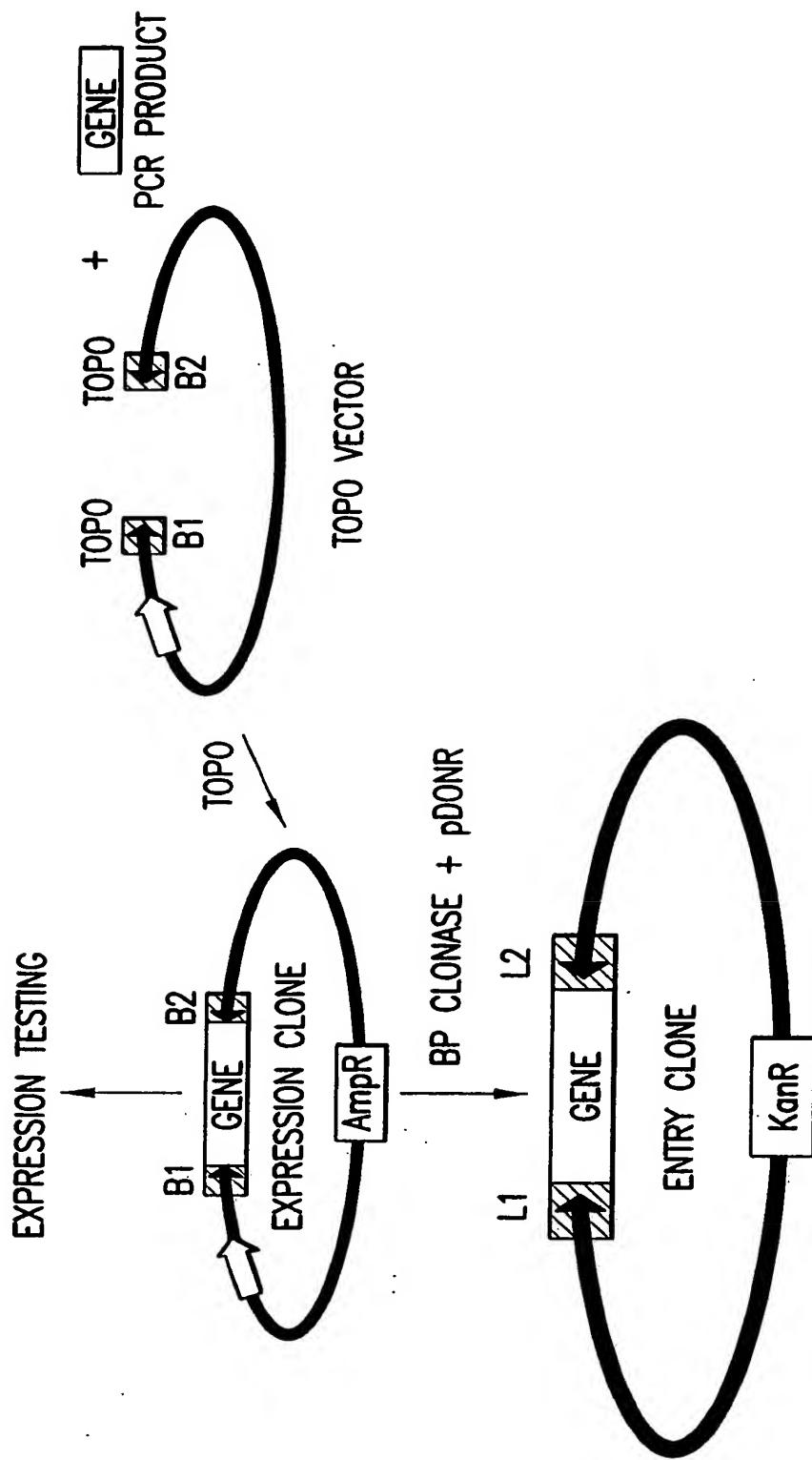


FIG. 17

MCS FOR pCDNAGW-DT(sc) AND pENTR-DT(sc)

L Y K K A G S A A G R A D P A F L Y K V
 ... TTG TAC AAA AAA GCA GGC TCC GCG GCC GCT CTC GAG AAA GGG GGC GAC CCA CCT TTC TTG TAC AAA GTG
BsrG I Xba I Asc I BsrG I


FIG. 18

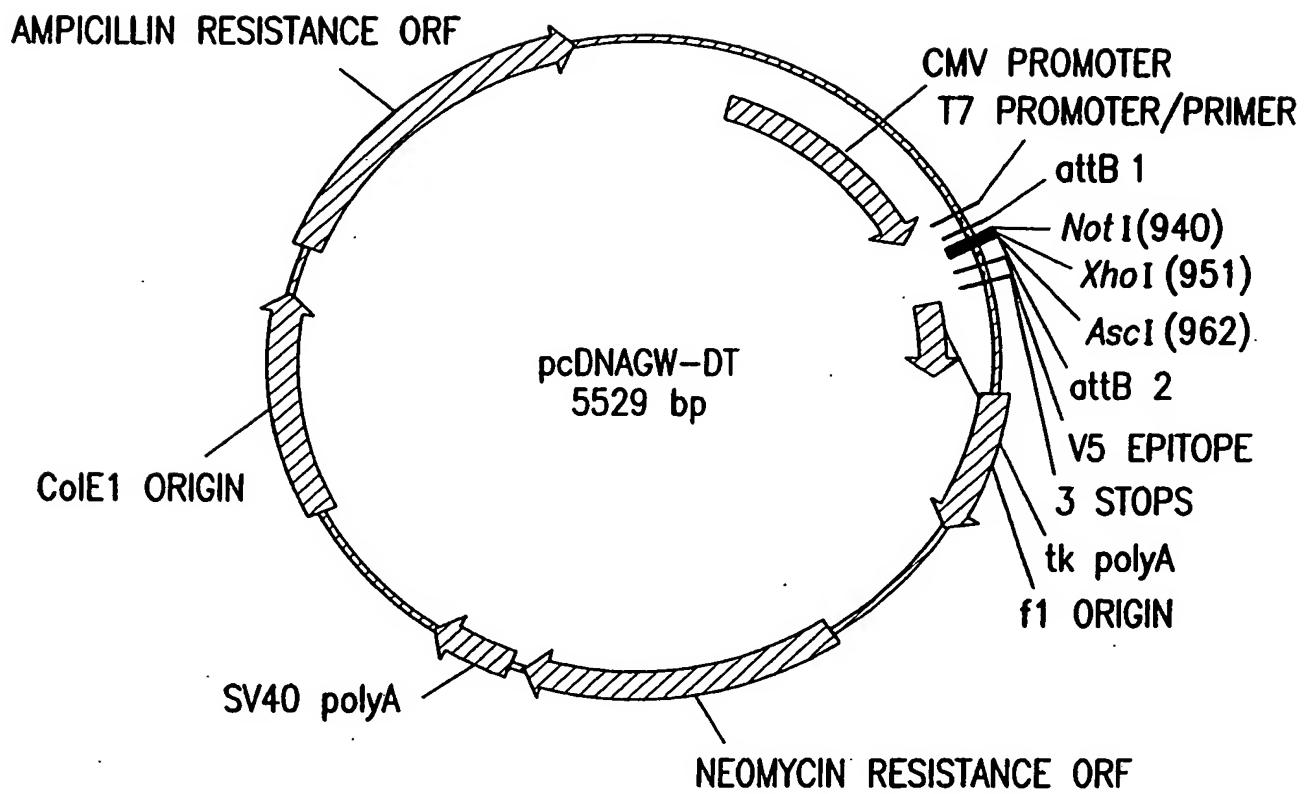


FIG. 19

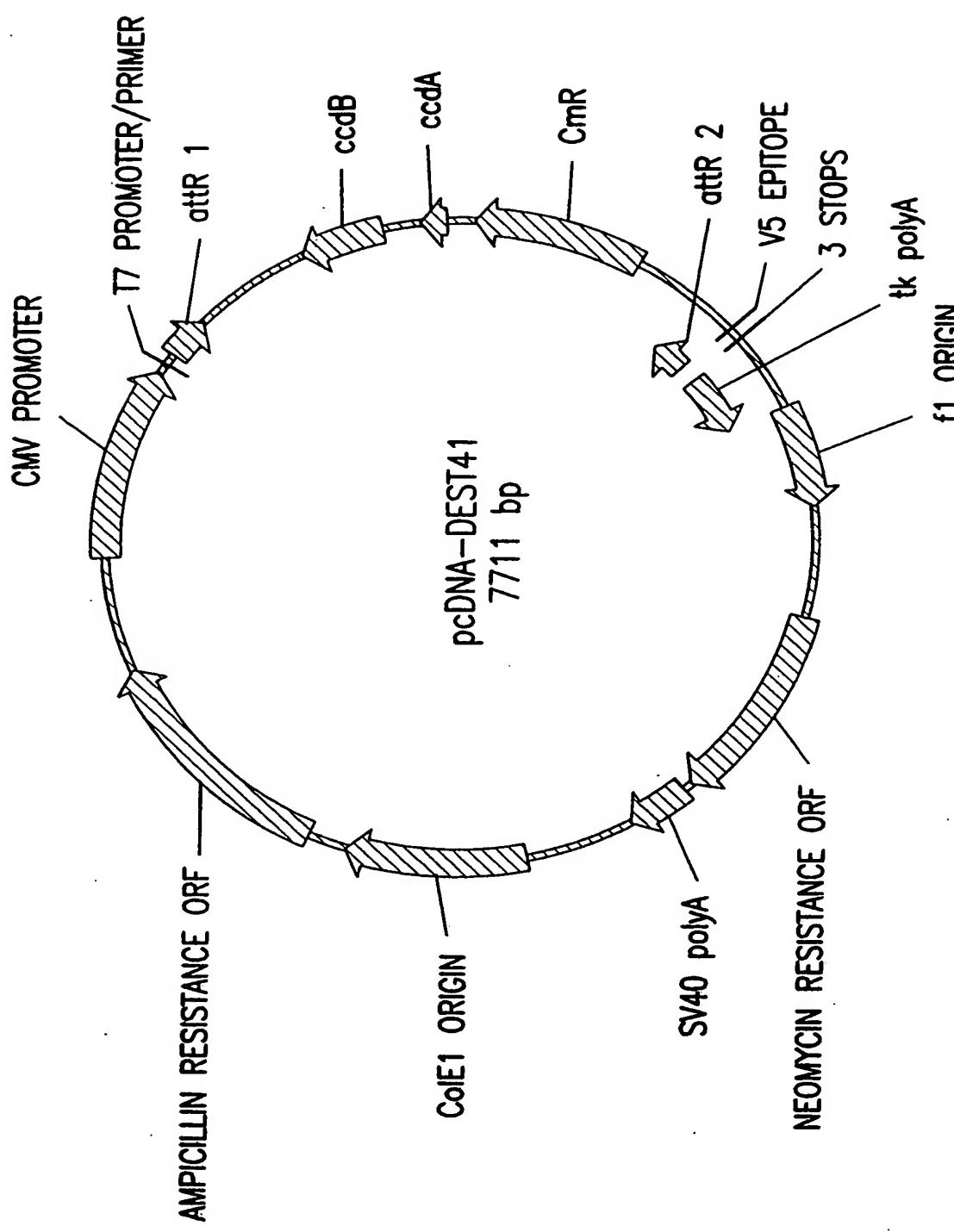


FIG. 20

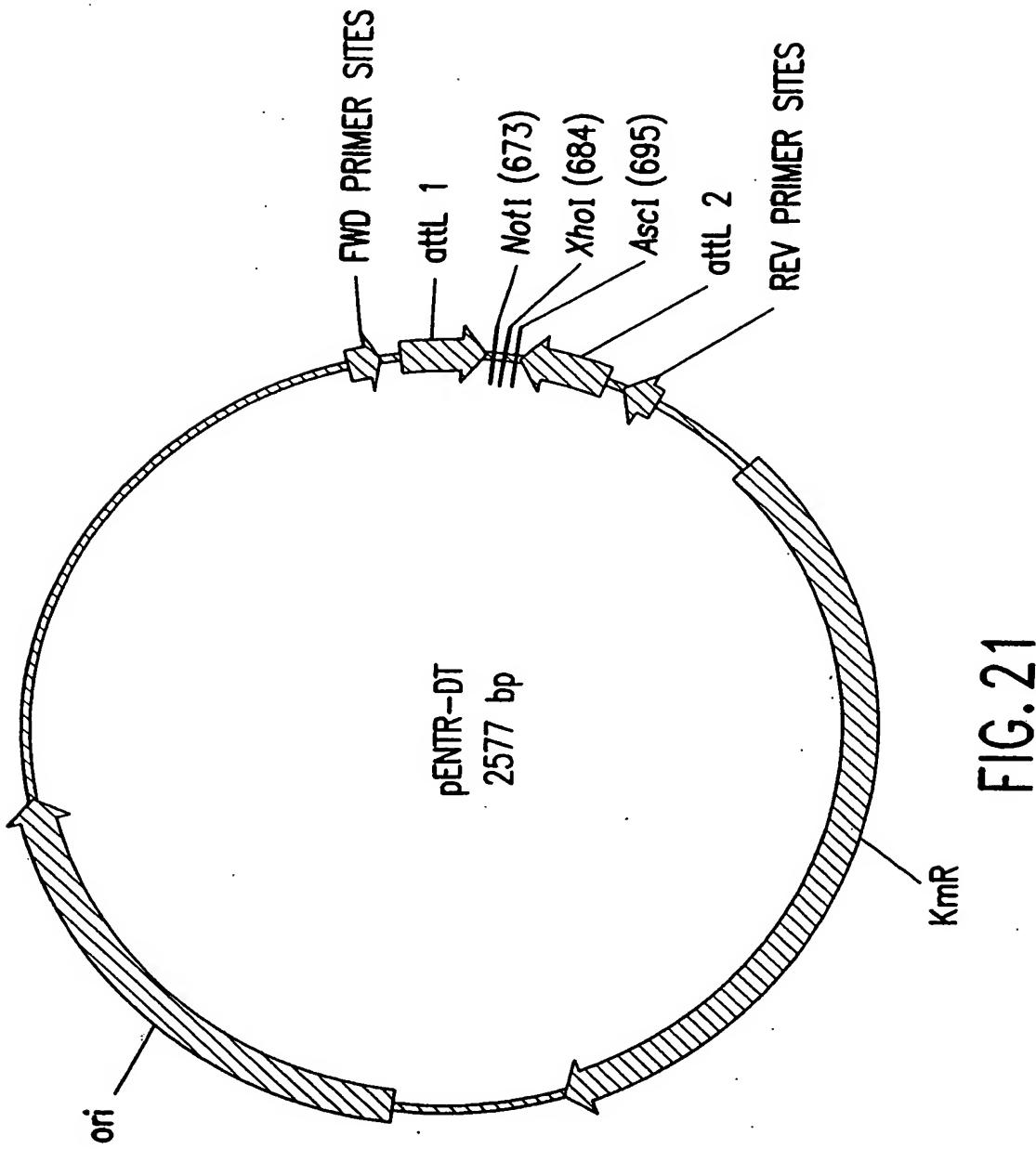


FIG. 21

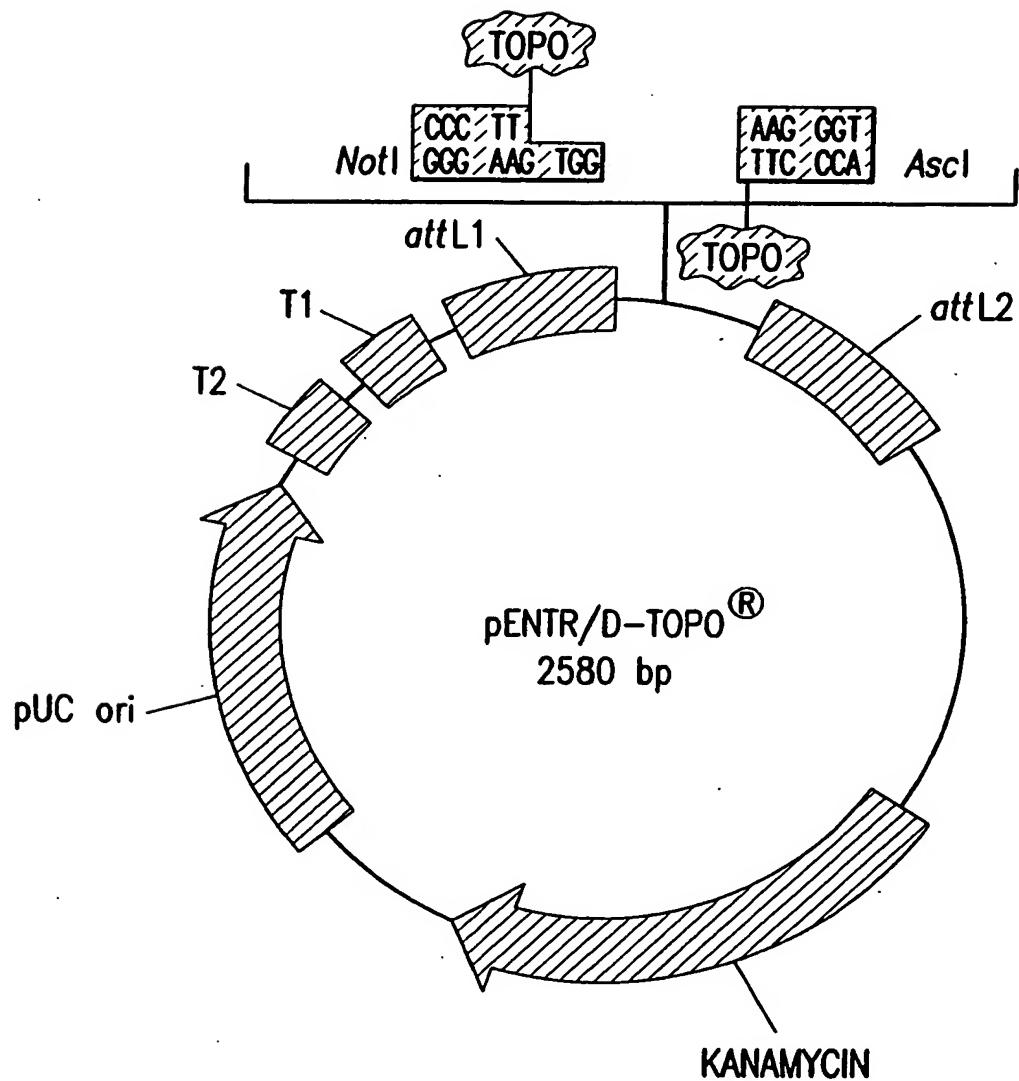


FIG. 22A

1 ctttcctgcg ttatcccttg attctgtgga taaccgtatt accgcctttg agttagctga
 61 taccgctcgc cgcaagccaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga
 121 gcccataa cgcaaacgc ctctccccgc gcgttggccg attcattaat gcagctggca
 181 cgacagggtt cccgactgga aagcggcgag tgagcgcaac gcaattaata cgcgtaccgc
 241 tagccaggaa gagttgttag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
 301 gtttcatgcc tggcagttt tggcggcggt cctgcccggc accctccggg cggttcttc
 361 acaacgttca aatccgctcc cggcgattt gtcctactca ggagagcggtt caccgacaaa
 421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
 481 gcagttccct actctcgctg taacgcttagc atggatgttt tccagtcac gacgttgtaa
 541 aacgacggcc agtctaagc tcgggccccca aataatgatt ttatTTTgac tgatagtgac
 601 ctgttcgttg caacaattt atgagcaatg ctttttata atgccaactt tgtacaaaaaa
 661 agcaggctcc gcggccggcc cttcaccatg nnnnnnnnna aggtggcg cggccaccca
 721 gcttcttgc acaaagtgg cattataaga aagcattgt tatcaatttgg tgcacacgaa
 781 caggtcaact tcagtcaaaa taaaatcatt atttgccatc cagctgatatt cccctatagt
 841 gagtcgtatt acatggtcat agctgttcc tggcagctct ggcccggtc tcaaaatctc
 901 tgatgttaca ttgcacaaga taaaatata tcacatgaa caataaaact gtctgcttac
 961 ataaaacagta atacaagggg tggtatgac catattcaac gggaaacgtc gaggccgcga
 1021 taaaattcca acatggatgc tgatttatgat ggtataaat gggctcgca taatgtcggg
 1081 caatcagggtg cgacaatcta tcgcttgcatt gggaaagcccg atgcgcaga gttgttctg
 1141 aaacatggca aaggtagcgt tgccaatgat gttacagatg agatggtcg actaaactgg
 1201 ctgacggaat ttatgcctct tccgaccatc aagcattttt tccgtactcc tcatgtatgca
 1261 tggtaactca ccactgcgtat ccccgaaaaa acagcattcc aggtatttgcg agaatatcct
 1321 gattcagggtg aaaatattgt tgatgcgtg gcagtgttcc tgcgcccgtt gcattcgatt
 1381 cctgtttgtt attgtcctt taacagcgat cgcgtatttgc gtctcgctca ggcgcaatca
 1441 cgaatgaata acgggttggc tgatgcgtat gatTTTgatg acgagcgtaa tggctggcct
 1501 gttgaacaag tctggaaaga aatgcataaa cttttgcatt tctcacccgaa ttcagtcgtc
 1561 actcatggtg atttctact tgataacctt attttgacg agggaaatt aataggttgt
 1621 attgatgttg gacgagtcgg aatcgcagac cgataccagg atcttgcatt cctatggAAC
 1681 tgcctcggtg agtttctcc ttcattacag aaacggcttt ttcaaaaata tggatttgat
 1741 aatcctgata tgaataaaatt gcagtttcat ttgatgctcg atgagttttt ctaatcagaa
 1801 ttgttaatt ggtttaaca ctggcagagc attacgtga cttgacgggaa cggcgcaagc
 1861 tcatgaccaa aatccctaa cgtgagttac gcgtcggttcc actgagcgtc agaccccgta
 1921 gaaaagatca aaggatctt ttgagatcct tttttctgc gcgtaatctg ctgcttgcac
 1981 aaaaaaaaaac caccgctacc agcgggtggtt tggccggcgtt atcaagagct accaactctt
 2041 tttccgaagg taactggctt cagcagagcg cagataccaa atactgtct tctagtgttag
 2101 ccgtagtttag gccaccactt caagaactct gtacgcaccgc ctacataacct cgctctgcta
 2161 atcctgttac cagtggctgc tgccagtgcc gataagtctg gtcttaccgg gttggactca
 2221 agacgatagt taccggataa ggcgcagcg tccggctgaa cgggggggttc gtgcacacag
 2281 cccagcttgg agcgaacgac ctacaccgaa ctgagatacc tacagcgtga gcattgagaa
 2341 agccacgc ttcccgaaagg gagaaggcg gacaggtatc cggtaagcggtt cagggtcgaa
 2401 acaggagagc gcacgaggaa gcttccaggaa gggaaacgcctt ggtatcttta tagtcctgtc
 2461 gggttcgcc acctctgact tgagcgctga tttttgtat gtcgtcagg ggggcggagc
 2521 ctatggaaaa acgcccggaa cgcggccctt ttacgggttcc tggccttttgc tggccttttgc
 2581 gtcacatgt t

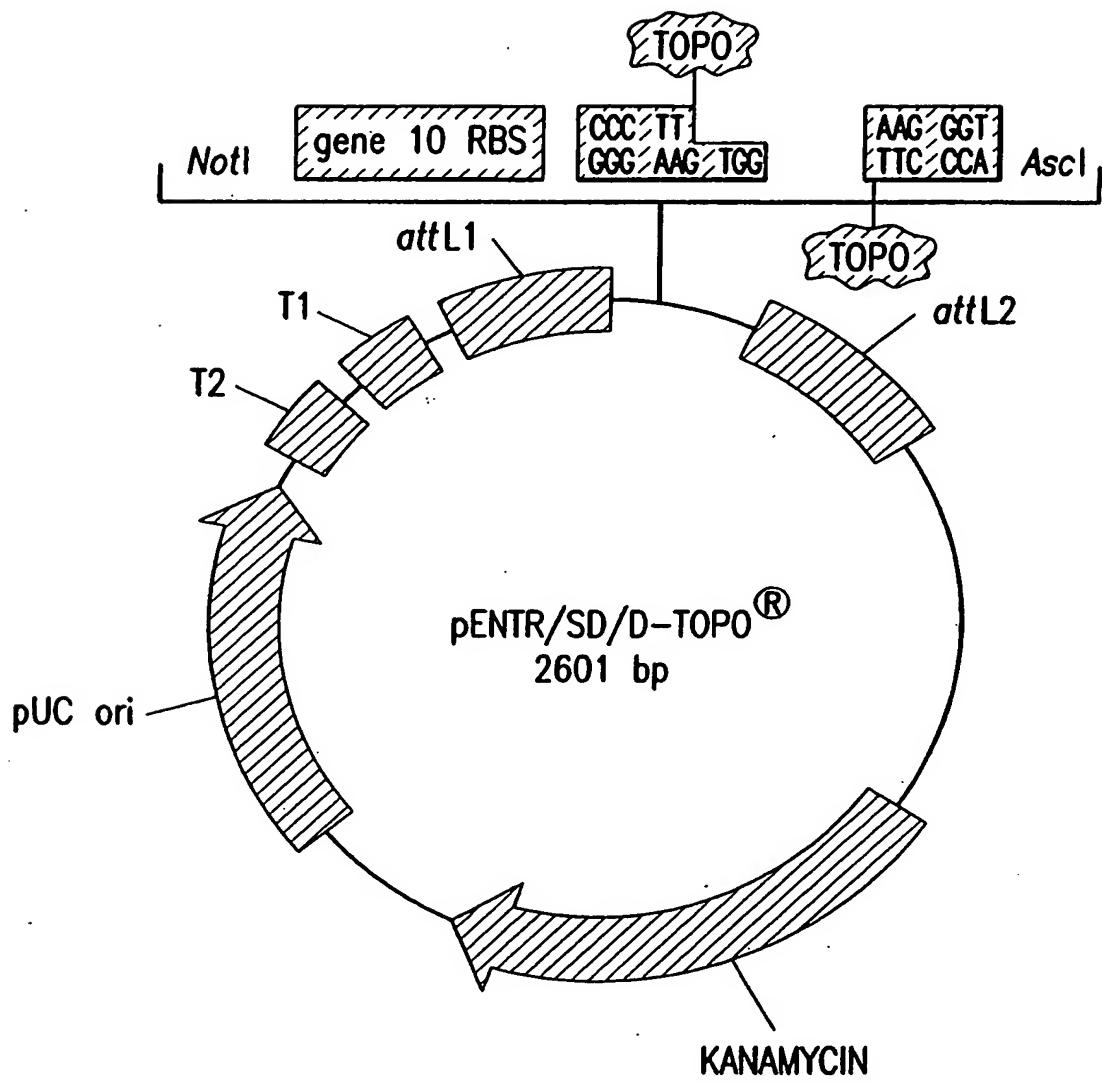


FIG. 23A

1 ctttcctgcttgc ttatcccgtt attctgtggta taaccgtatt accgcctttt agtgagctga
 61 taccgctcgcc cgcaagccgaa cgaccgagcg cagcagtcgtca gtgagcgagg aagcggaaga
 121 gcgcggccata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
 181 cgacagggtt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
 241 tagccaggaa gagttttagt aaacgcaaaa aggccatccg tcagggatggc cttctgctta
 301 gttttagtgc tggcagttt tggcggcggt cctgcccgc accctccggg ccgttgcctt
 361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcggtt caccgacaaa
 421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttgcgttttat ttgatgcctg
 481 gcagttccct actctcgctg taacgcttagc atggatgttt tcccagtcac gacgttgtaa
 541 aacgacggcc agtcttaagc tcggggccca aataatgatt ttatgttgc tgatagtgc
 601 ctgttcgttt caacaaattt atgagcaatg ctttttata atgccaactt tgtacaaaaaa
 661 agcaggctcc gcggccgcct tgtttaactt taagaaggag cccttcaccn nnnnnaaggg
 721 tggcgcgcgc gaccagttt tcttgtacaa agttggcatt ataagaaagc attgcttatac
 781 aattttgttgc aacgaacagg tcactatcag tcaaaataaa atcattattt gccatccagc
 841 tgatatatcccc tatagttagt cgtattacat ggtcatagct gttcctggc agctctggcc
 901 cgtgtctcaa aatctctgat gttacattgc acaagataaa aatatatcat catgaacaat
 961 aaaactgtct gcttacataa acagtaatac aagggggttt atgagccata ttcaacggga
 1021 aacgtcgagg ccgcgattaa attccaaacat ggatgctgat ttatatgggt ataaatgggc
 1081 tcgcpataat gtcgggcaat caggtgcgc aatctatcgc ttgtatggga agcccgatgc
 1141 gccagagttt tttctgaaac atggcaaaagg tagcgttggc aatgtatgtt cagatgagat
 1201 ggtcagacta aactggctga cggaaatttgc gcctcttccg accatcaagc attttatccg
 1261 tactcctgat gatgcattgt tactcaccac tgcgatcccc gggaaaaacag cattccagggt
 1321 attagaagaa tatttcgtt caggtaaaaa tattttgtat ggcgtggcag tggtcctgcg
 1381 ccgttgcatttcgatttgc tttgtatttgc tccttttaac agcgatcgcc tatttcgtct
 1441 cgctcaggcg caatcacgaa tgaataacgg tttgggttgc gcgagtgtt ttgatgacga
 1501 gcgtaatggc tggcctgtt aacaagtctg gaaagaaaatg cataaaacttt tgccattctc
 1561 accggattca gtcgtcactc atgggtattt ctcacttgc aaccttattt ttgacgaggg
 1621 gaaatttataa gtttggatttgc atgttggacg agtcggaaatc gcagaccgat accaggatct
 1681 tgccatccta tggaaactgcc tcgggtgat ttctccttca ttacagaaac ggcttttca
 1741 aaaatatggt attgataatc ctgatatgaa taaattgcag tttcattttgc tgctcgatga
 1801 gtttttctaa tcagaattgg ttaattgggtt gtaacactgg cagacgatcc cgtctgactt
 1861 acgggacggc gcaagctcat gacaaaatc ccttaacgtt agttacgctt cgttccactg
 1921 agcgtcagac cccgtagaaa agatcaaagg atcttcttgc gatcctttt ttctgcgcgt
 1981 aatctgtgc ttgcaaaacaa aaaaaccacc gtcaccagcg gtgggttgc tgccggatca
 2041 agagctacca actcttttc cgaaggtaac tggcttcagc agagcgacgata taccacaaatc
 2101 tgcccttcta gtgttagccgt agttaggcca ccacttcaag aactctgttag caccgcctac
 2161 atacctcgct ctgctaattcc tggttaccatgt ggctgctgcc agtggcgata agtcgtgtct
 2221 taccgggttgc gactcaagac gatagttacc ggataaggcg cagcggtcgg gctgaacggg
 2281 gggttcgtgc acacagccca gcttggagcg aacgacctac accgaactga gataacctaca
 2341 gcgtgagcat tgagaagcg ccacgcttcc cgaaggggaga aaggcggaca ggtatccggt
 2401 aagcggcagg gtcggaaacag gagagcgac gagggagctt ccagggggaa acgcctggta
 2461 tctttatagt cctgtcggtt ttcgcccacct ctgacttgcg cgtcgatttt ttttgcgtt
 2521 gtcagggggg cggagccat ggaaaaaacgc cagcaacgctg gccttttac gttcctggc
 2581 cttttgcttgc cttttgctc acatgtt

FIG.23B

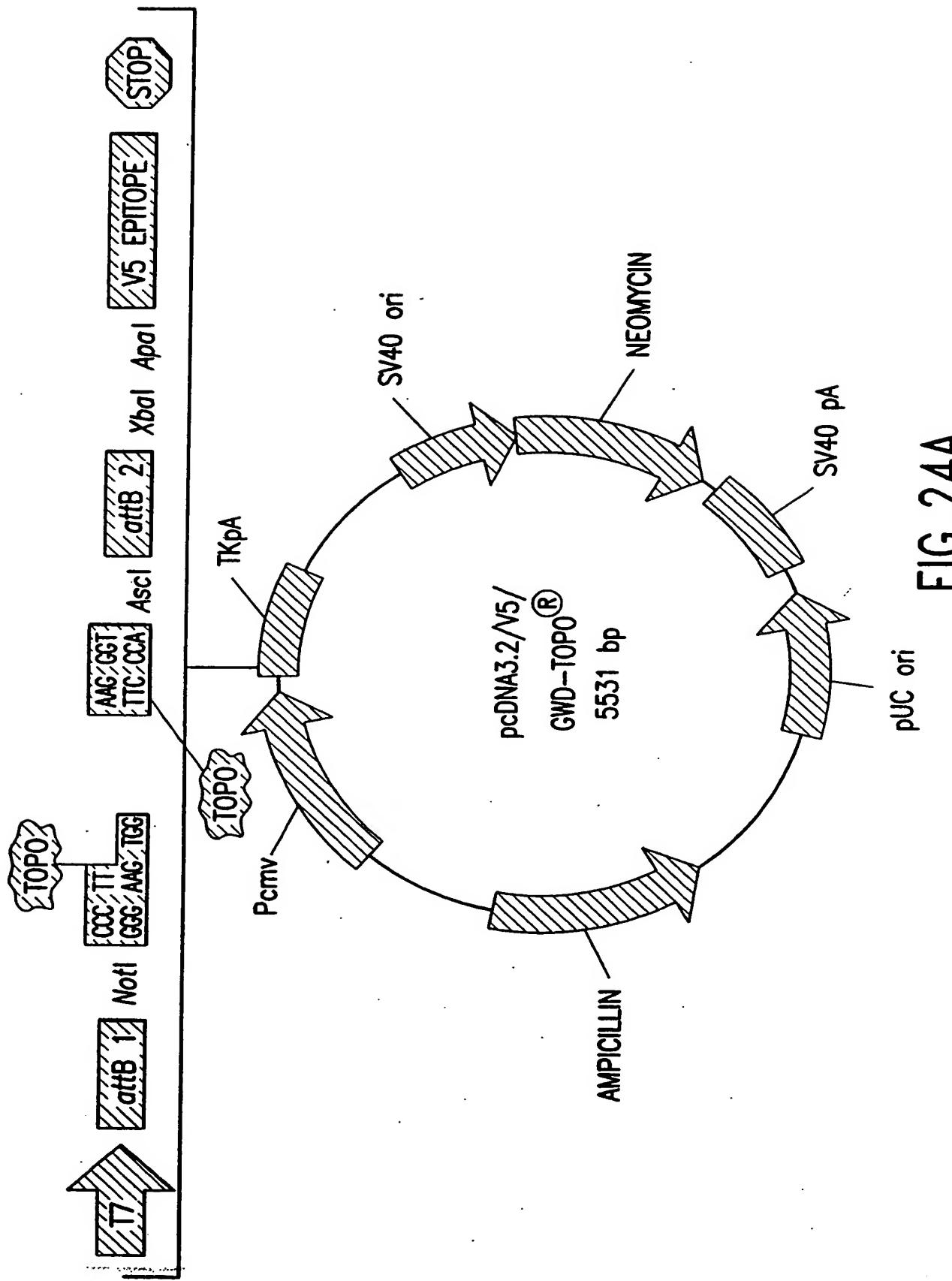


FIG. 24A

1 gacggatcgg gagatctccc gatcccstat ggtcgactct cagtacaatc tgctctgatg
 61 ccgcatagtt aagccagtt ctgctccctg ctttgtgtt ggaggcgct gtagtagtgcg
 121 cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatt aagaatctgc
 181 ttagggtag gcgtttgcg ctgcttcgcg atgtacggc cagatatacg cggtgacatt
 241 gattattgac tagtattaa tagtaatcaa ttacgggtc attagttcat agcccatata
 301 tggagttccg cgttacataa cttacgtaa atggcccgcc tggctgaccg cccaacgacc
 361 cccgcccatt gacgtcaata atgacgtatg ttccccatagt aacgccaata gggactttcc
 421 attgacgtca atgggtggac tatttacggt aaactgccc cttggcagta catcaagtgt
 481 atcatatgcc aagtacgccc cctattgcg tcaatgacgg taaatggccc gcctggcatt
 541 atgcccagta catgaccta tgggactttc ctacttggca gtacatctac gtattagtca
 601 tcgctattac catggtgatg cggtttggc agtacatcaa tggcgtgga tagcggtttgc
 661 actcacggg atttcaagt ctccacccca ttgacgtcaa tggaggttgc ttttggcacc
 721 aaaatcaacg ggactttcca aatgtcgta acaactccgc cccattgacg caaatggcg
 781 gtaggcgtgt acgggtggag gtctatataa gcagagctt ctgctaact agagaaccca
 841 ctgcttactg gcttatcgaa attaatacga ctcactatacg ggagacccaa gctggctagt
 901 taagctatca acaagttgt aaaaaaaaaaagg aggctccgc gccgcccattt caccatgnnn
 961 nnnnnnaagg gtggcgcgc cgacccagct ttcttgcata aagtgggtga tctagaggc
 1021 ccgcgttgc aaggtaagcc tatccctaac cctctcctcg gtctcgattc tacgcgtacc
 1081 ggttagtaat gagtttaaac gggggaggct aactgaaaaca cggaaggaga caataccgga
 1141 aggaacccgc gctatgacgg caataaaaaag acagaataaa acgcacgggt gttgggtcgt
 1201 ttgttcataa acgcgggggtt cggtcccagg gctggcactc tgtcgatacc ccaccgagac
 1261 cccattgggg ccaatacgcc cgcgttctt cttttcccc accccacccc ccaagttcgg
 1321 gtgaaggccc agggctcgca gccaacgtcg gggcggcagg ccctgccata gcagatctgc
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 1441 gtgtgggtgt tacgcgcagc gtgaccgcta cacttgcagc cgcctagcgc cccgctcctt
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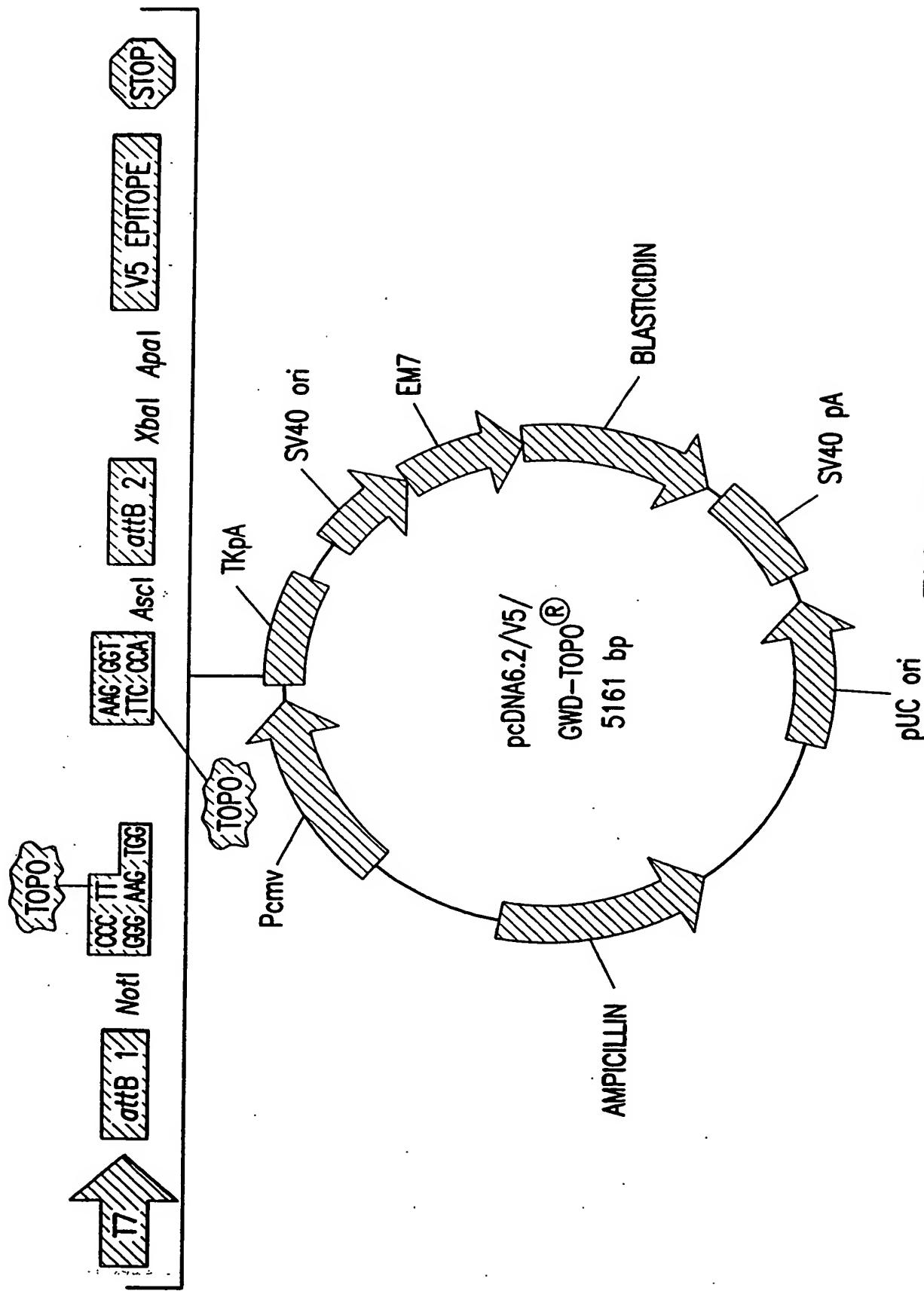


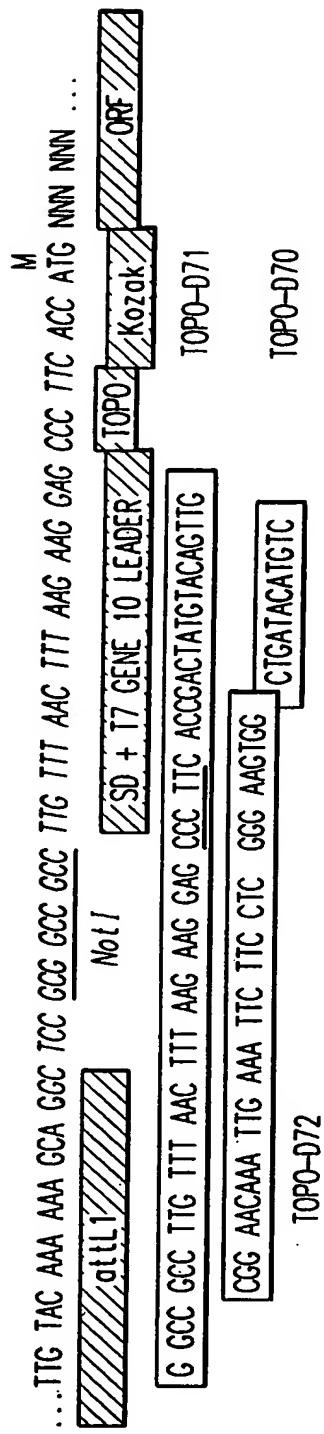
FIG. 25A

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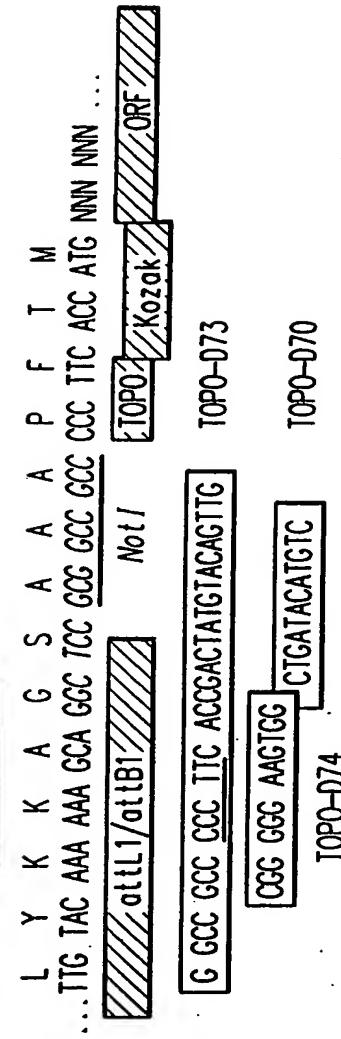
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 5161 gcccacctgac gtc

FIG.25C

pENTR/SD-dTOPO: 5' END



pENTR-dTOPO AND pcDNAGW-dTOPO: 5' END



pENTR/SD-dTOPO, pENTR-dTOPO, AND pcDNAGW-dTOPO: 3' END

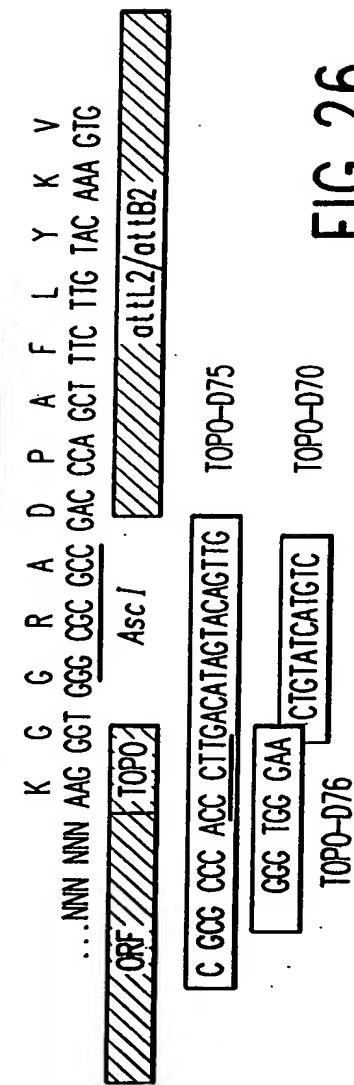


FIG. 26

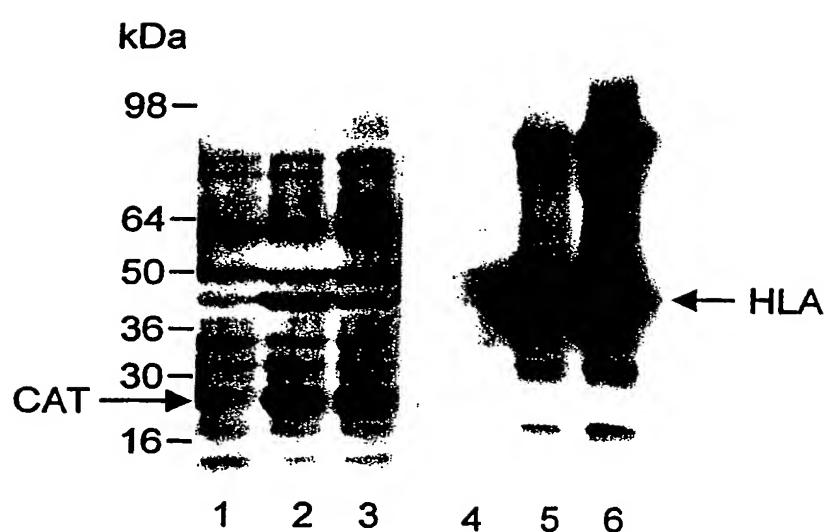


FIG.27

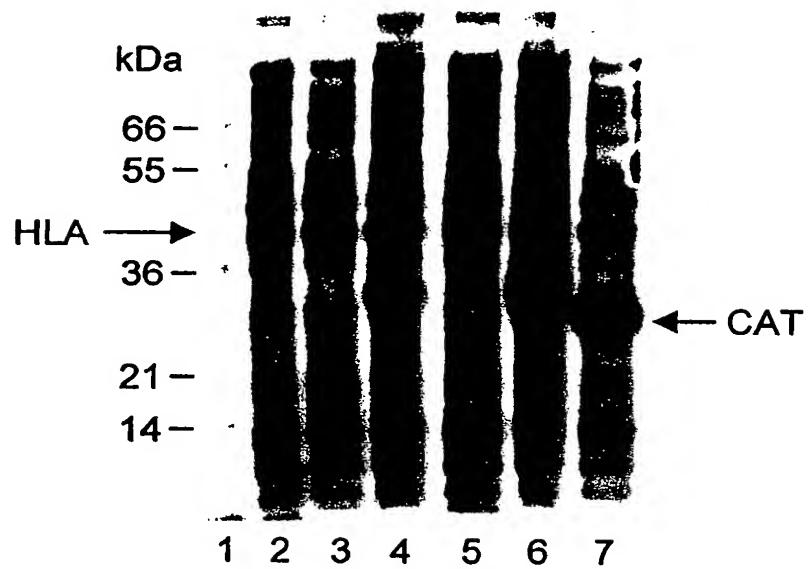


FIG.28

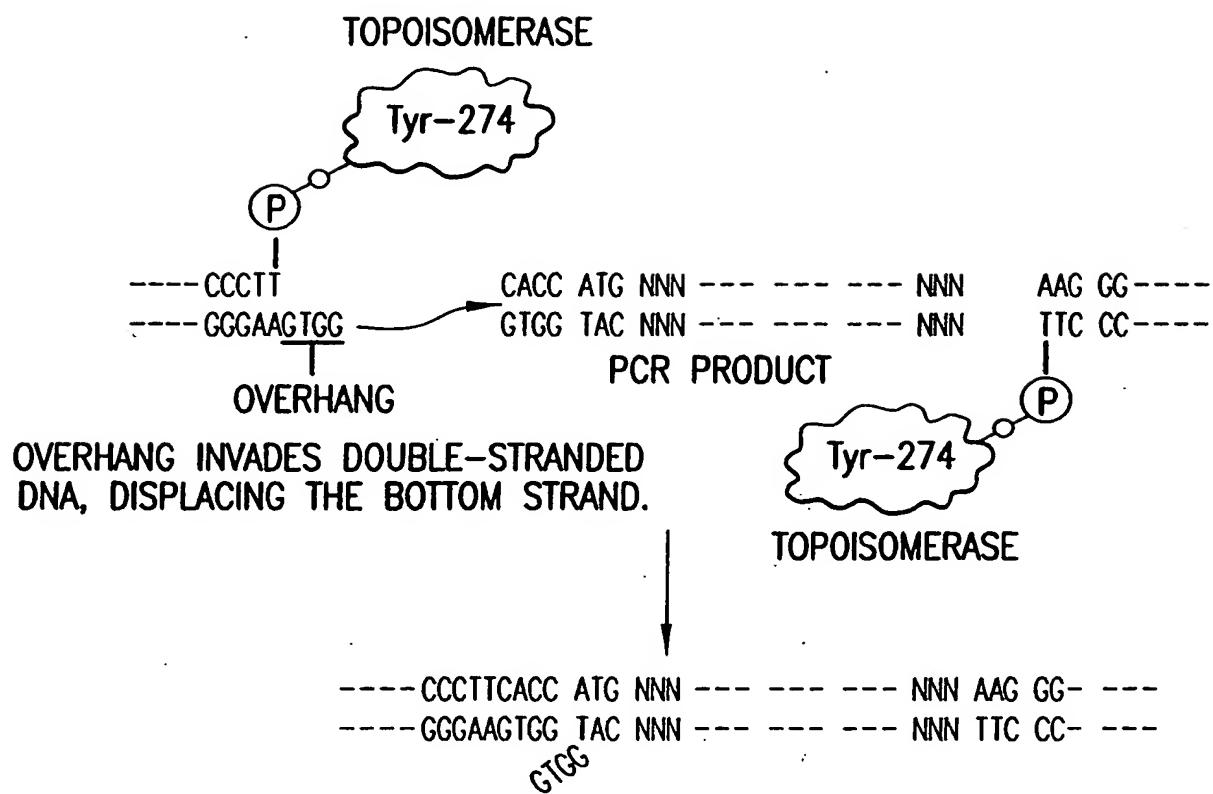


FIG. 29

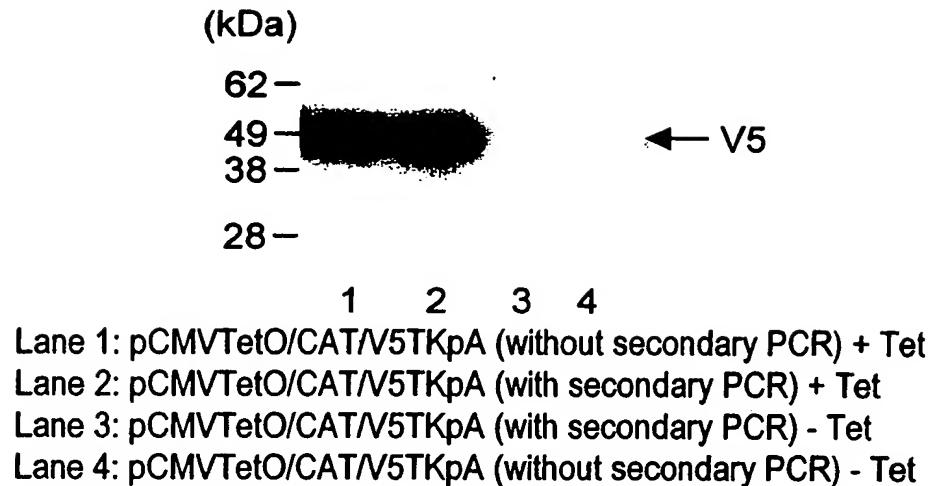
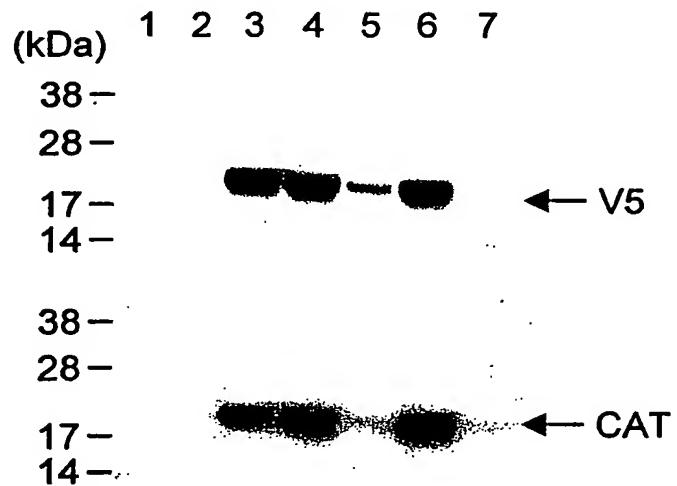
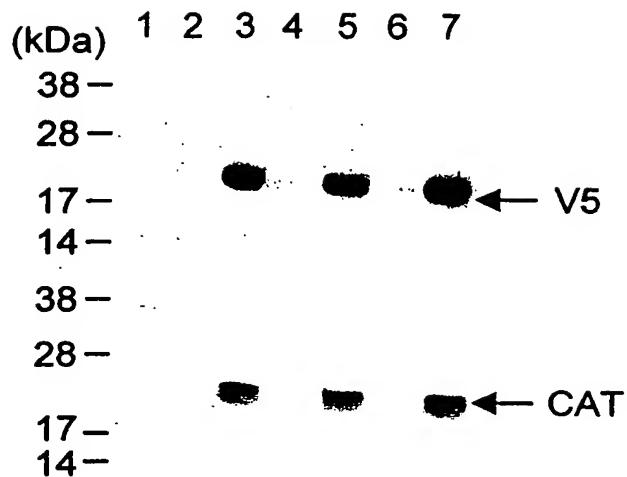


FIG.30A



Lane 1: TRex-CHO Cells + Tet
Lane 2: without secondary PCR (with purified CAT) - Tet
Lane 3: without secondary PCR (with purified CAT) + Tet
Lane 4: without secondary PCR (with unpurified CAT) + Tet
Lane 5: without secondary PCR (with unpurified CAT) -Tet
Lane 6: with secondary PCR + Tet
Lane 7: with secondary PCR - Tet

FIG.30B



Lane 1: TRex-293 Cells + Tet
Lane 2: without secondary PCR (with purified CAT) - Tet
Lane 3: without secondary PCR (with purified CAT) + Tet
Lane 4: without secondary PCR (with unpurified CAT) - Tet
Lane 5: without secondary PCR (with unpurified CAT) +Tet
Lane 6: with secondary PCR - Tet
Lane 7: with secondary PCR + Tet

FIG.30C



Lane 1: negative control; lanes 2-11: test clones; M: 500 bp marker

FIG.31

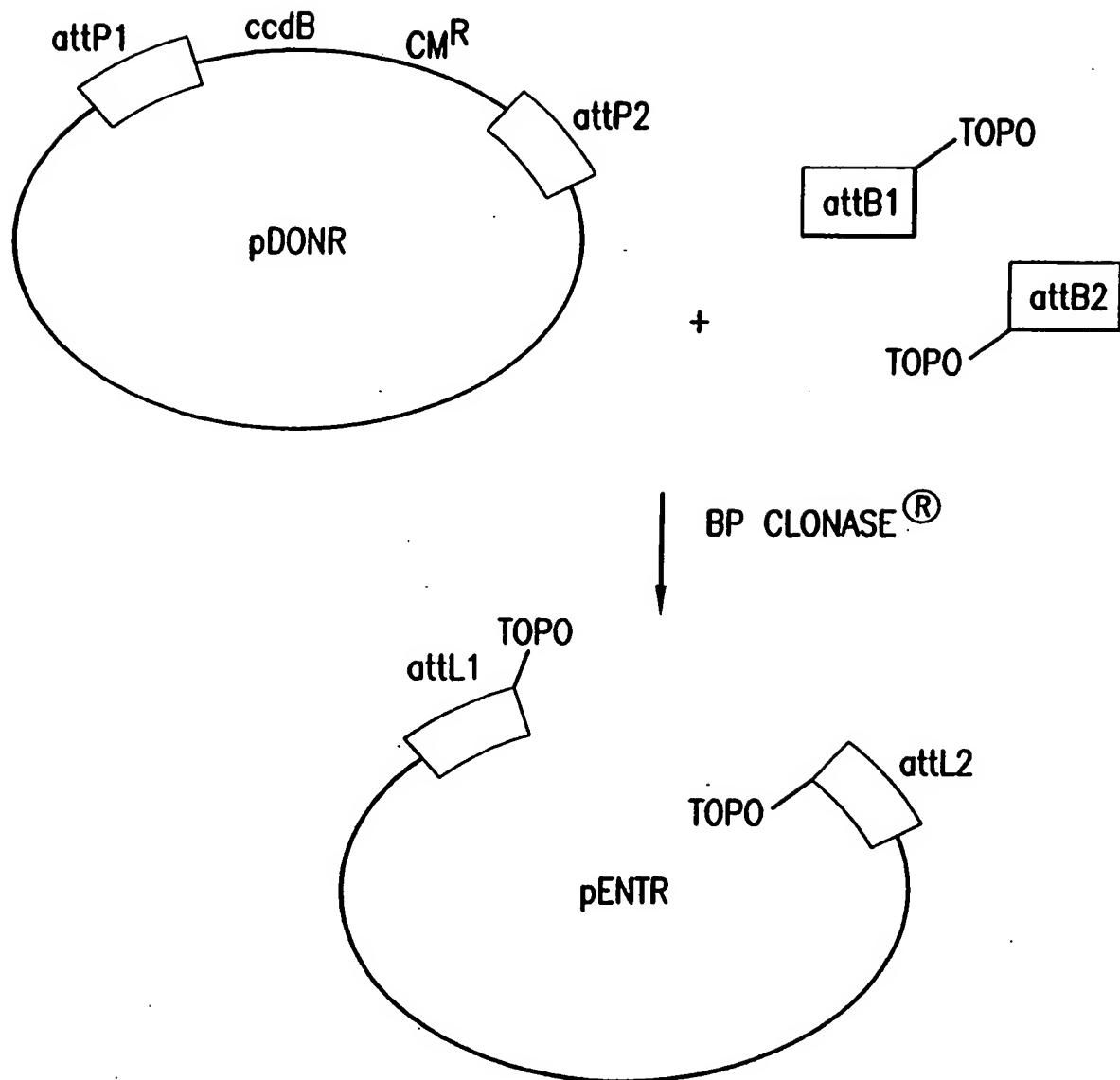
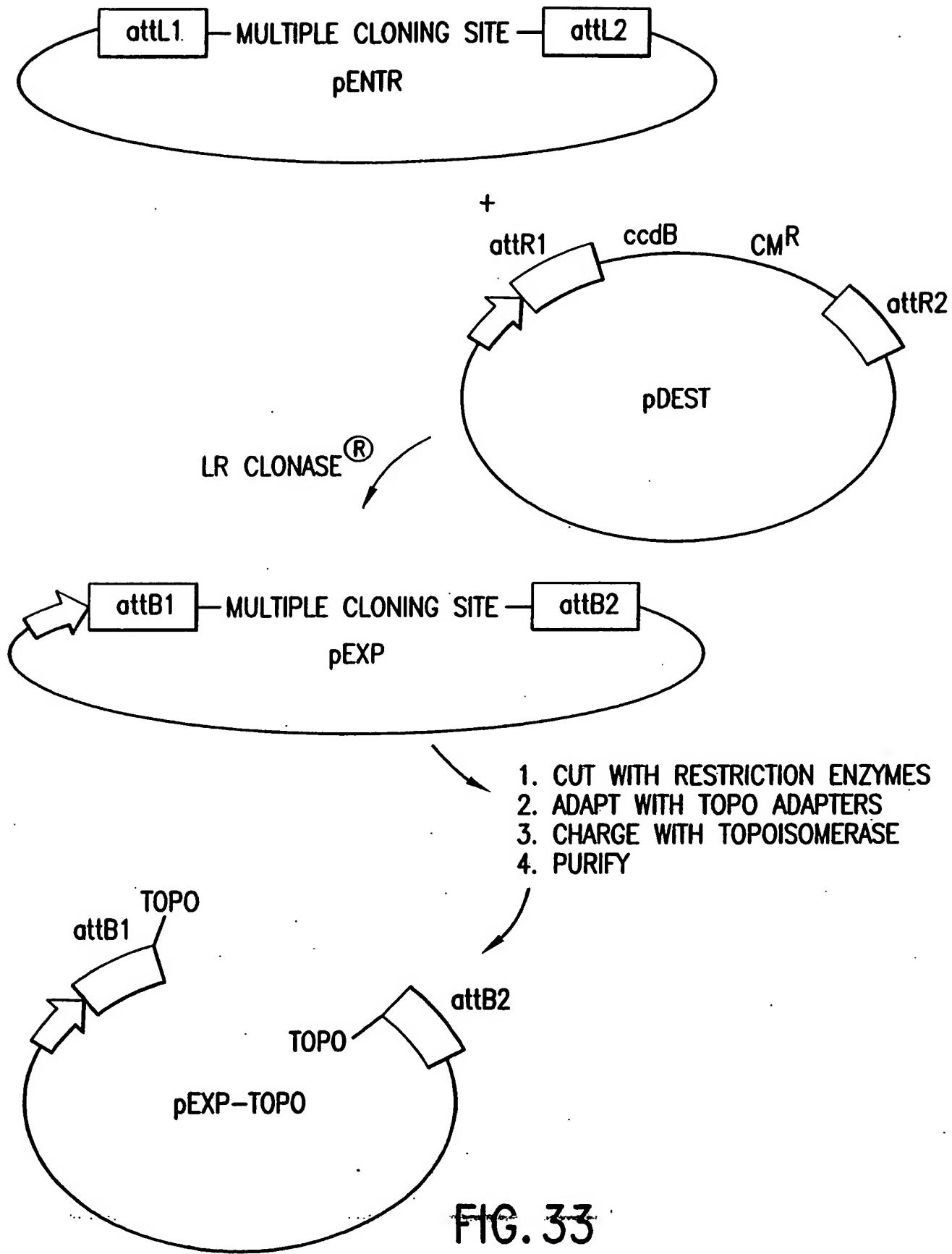


FIG. 32

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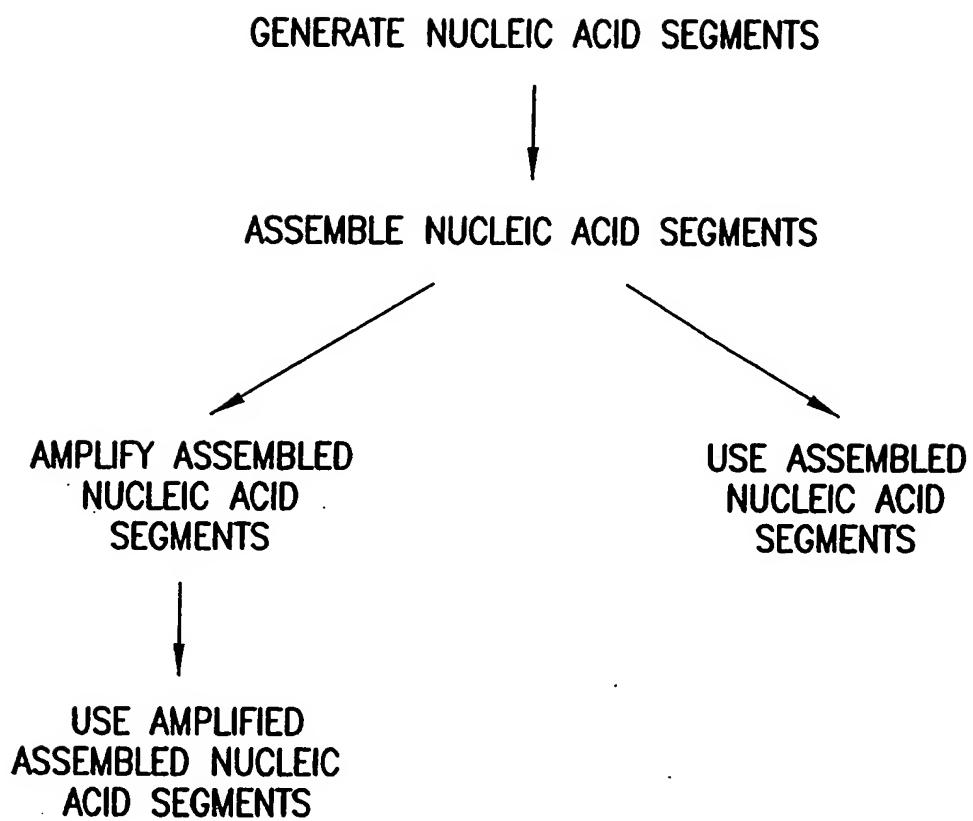


FIG. 34

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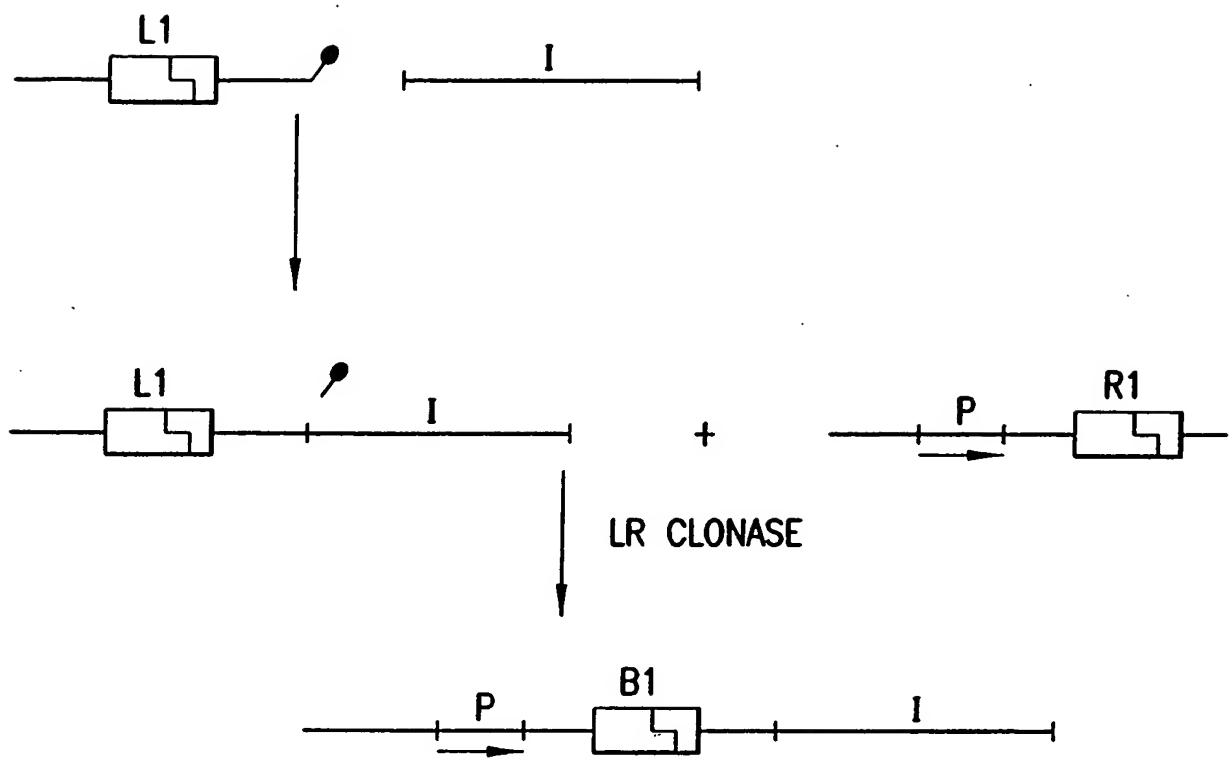


FIG. 35

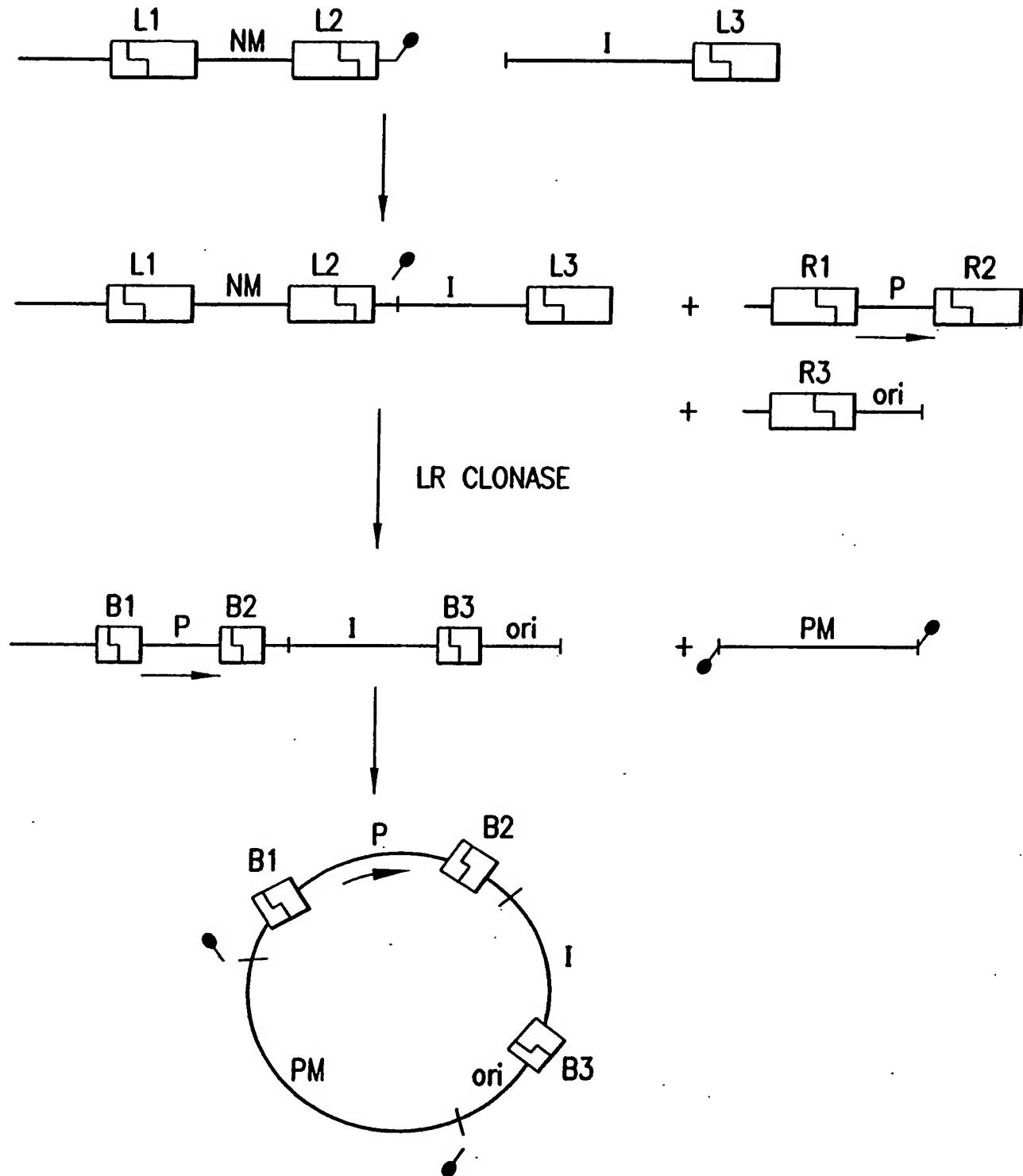


FIG. 36

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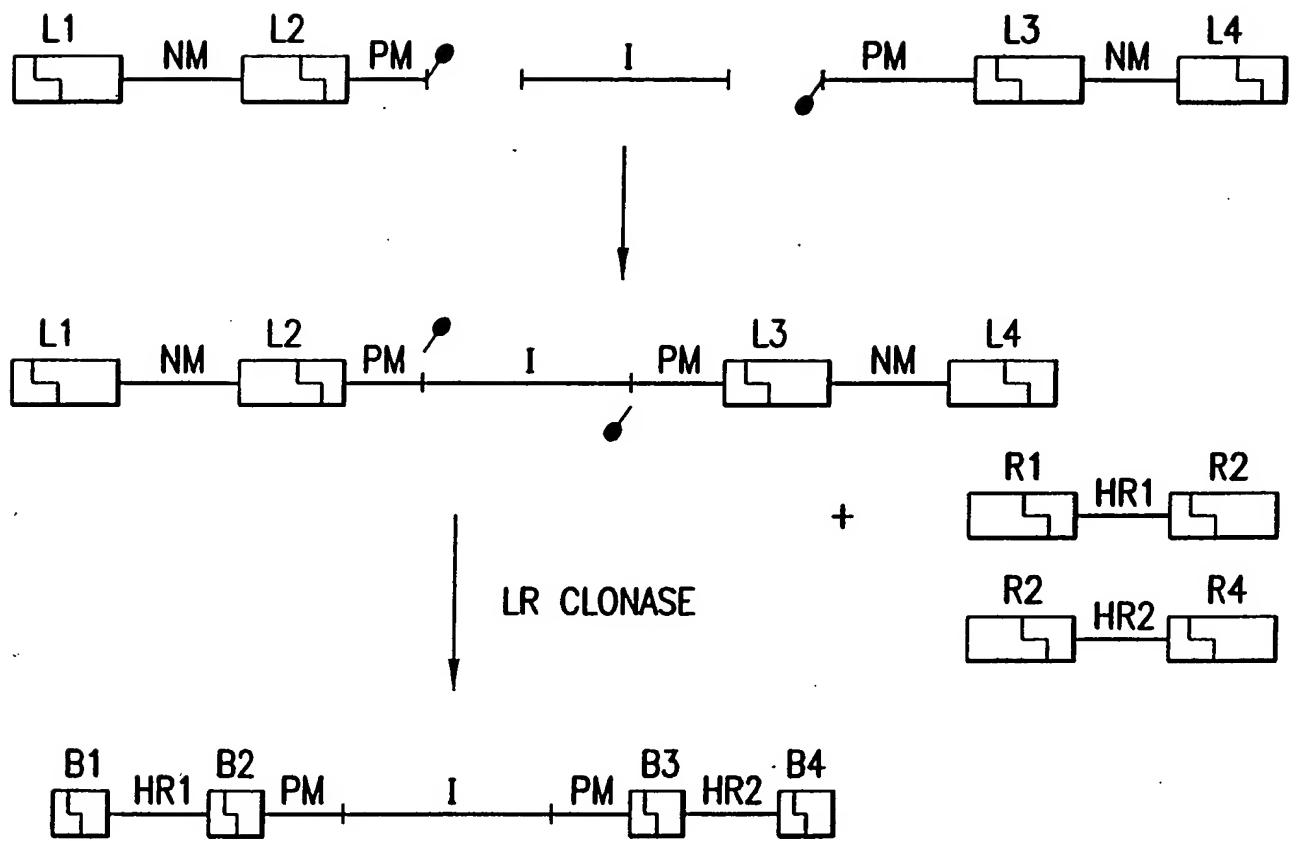


FIG. 37

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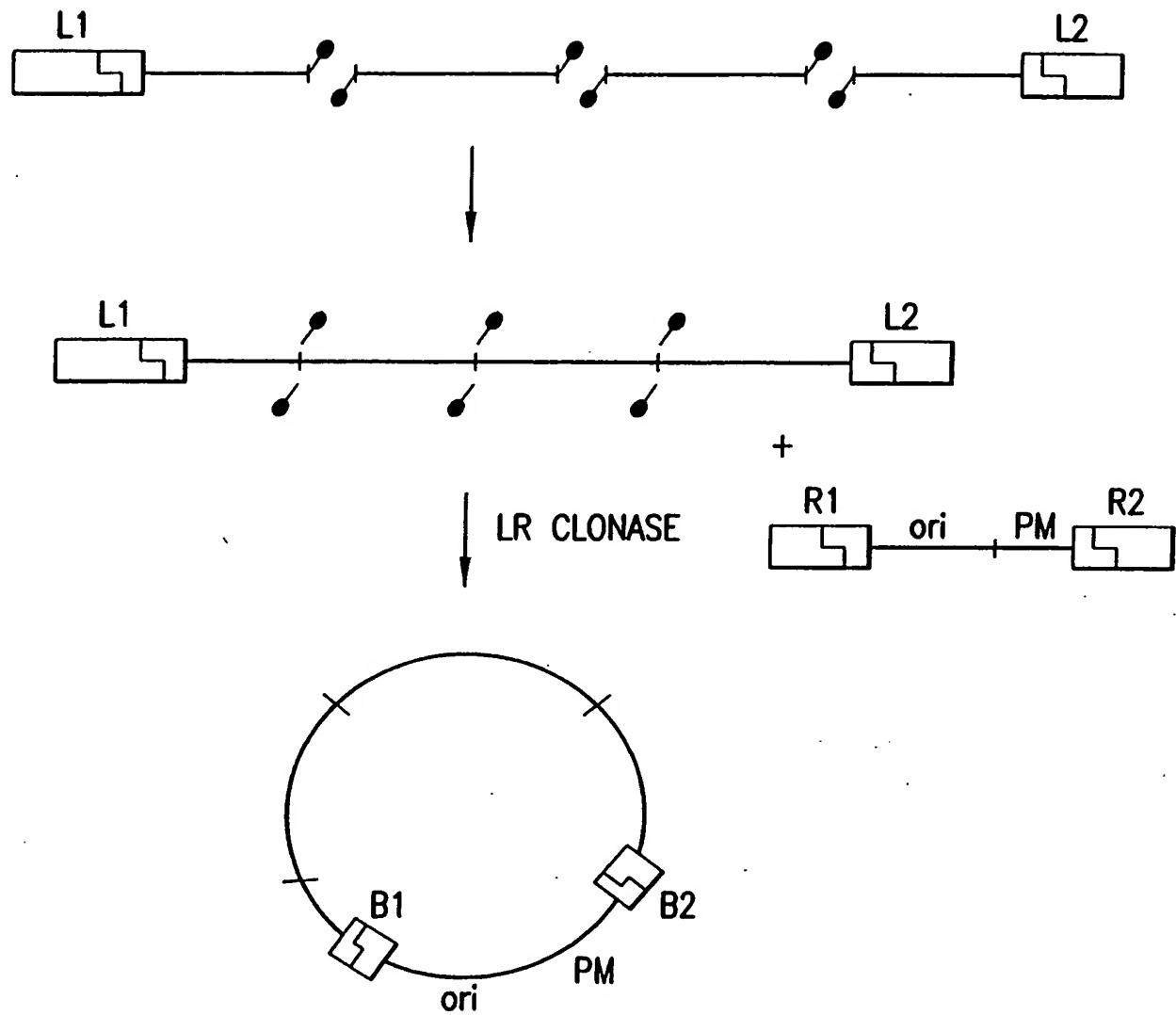


FIG. 38

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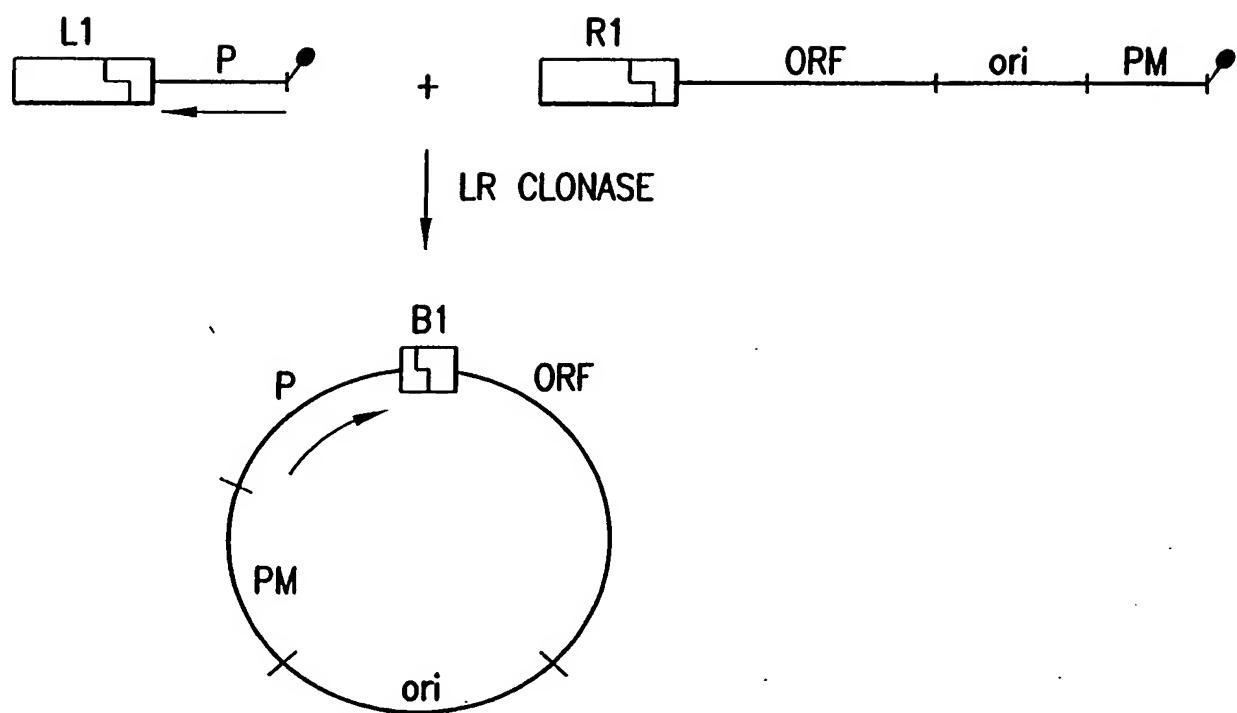


FIG. 39

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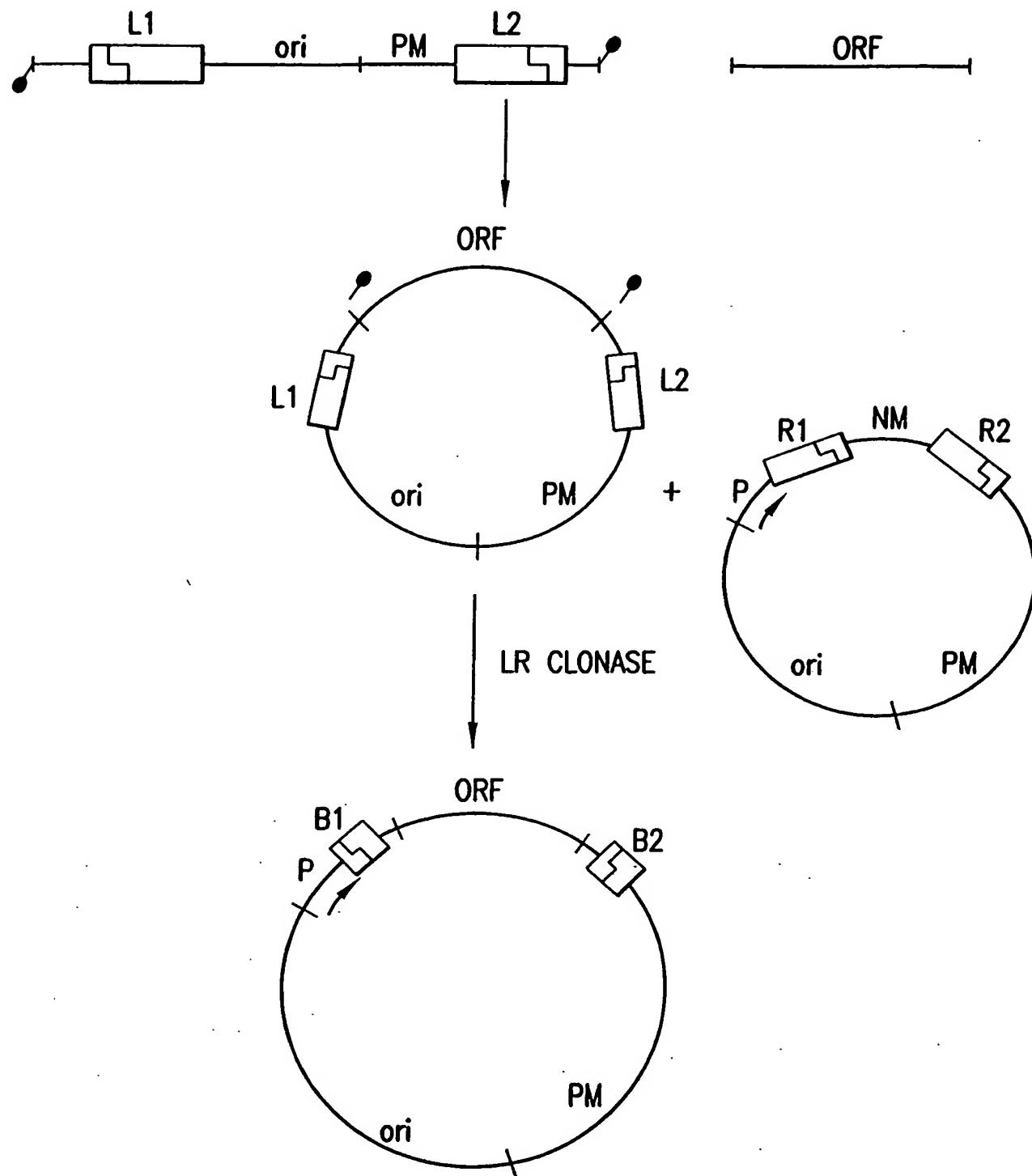
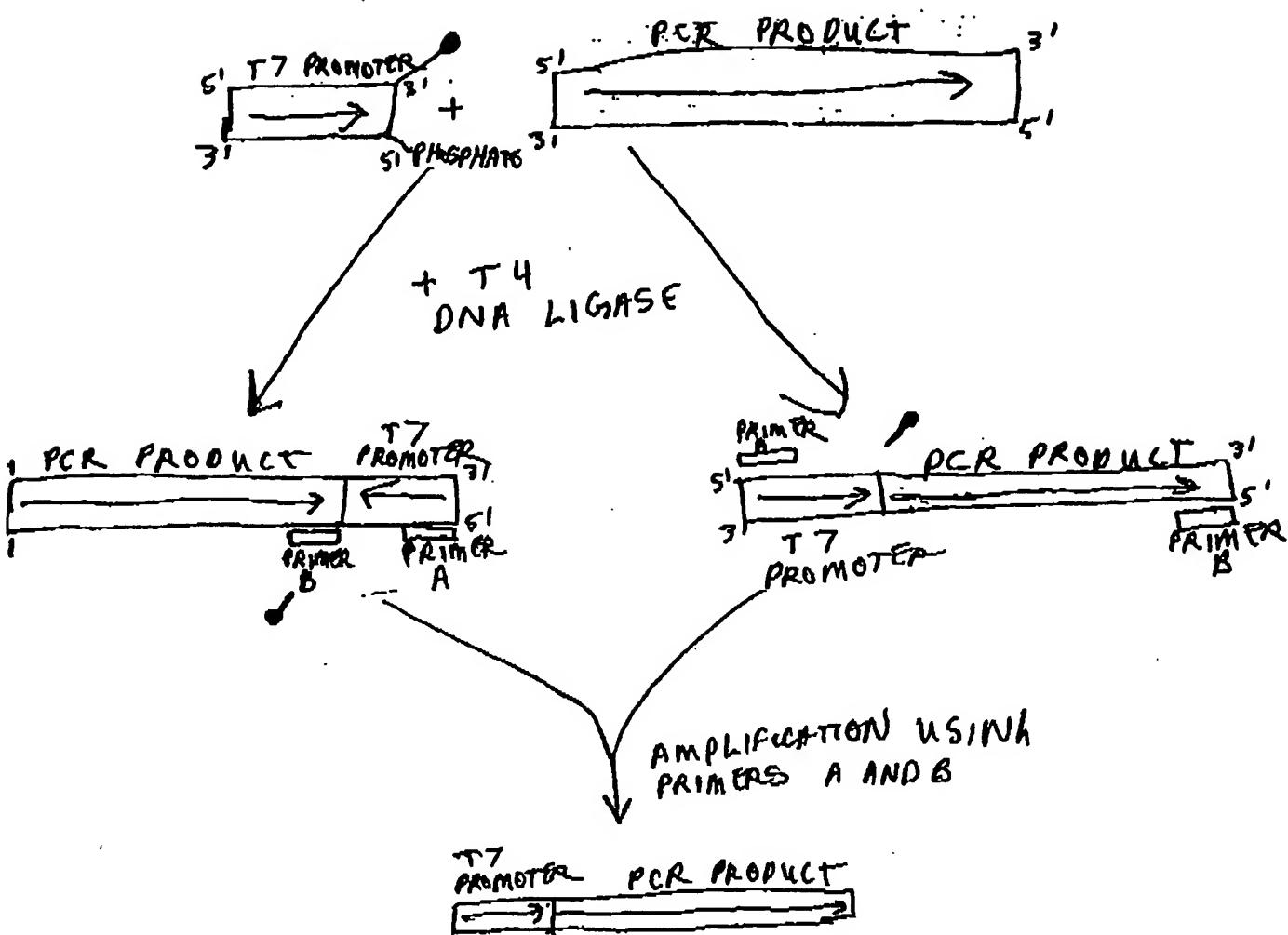


FIG. 40

FIGURE 4.1



A.

T7 promoter TOPO
5' pGACTCGTAATACGACTCACTATAGGGCCCTT 3'
3' AAAAAAAAAAACTGAGCATTATGCTGAGTGATATCCCGGGAp 5'

B.

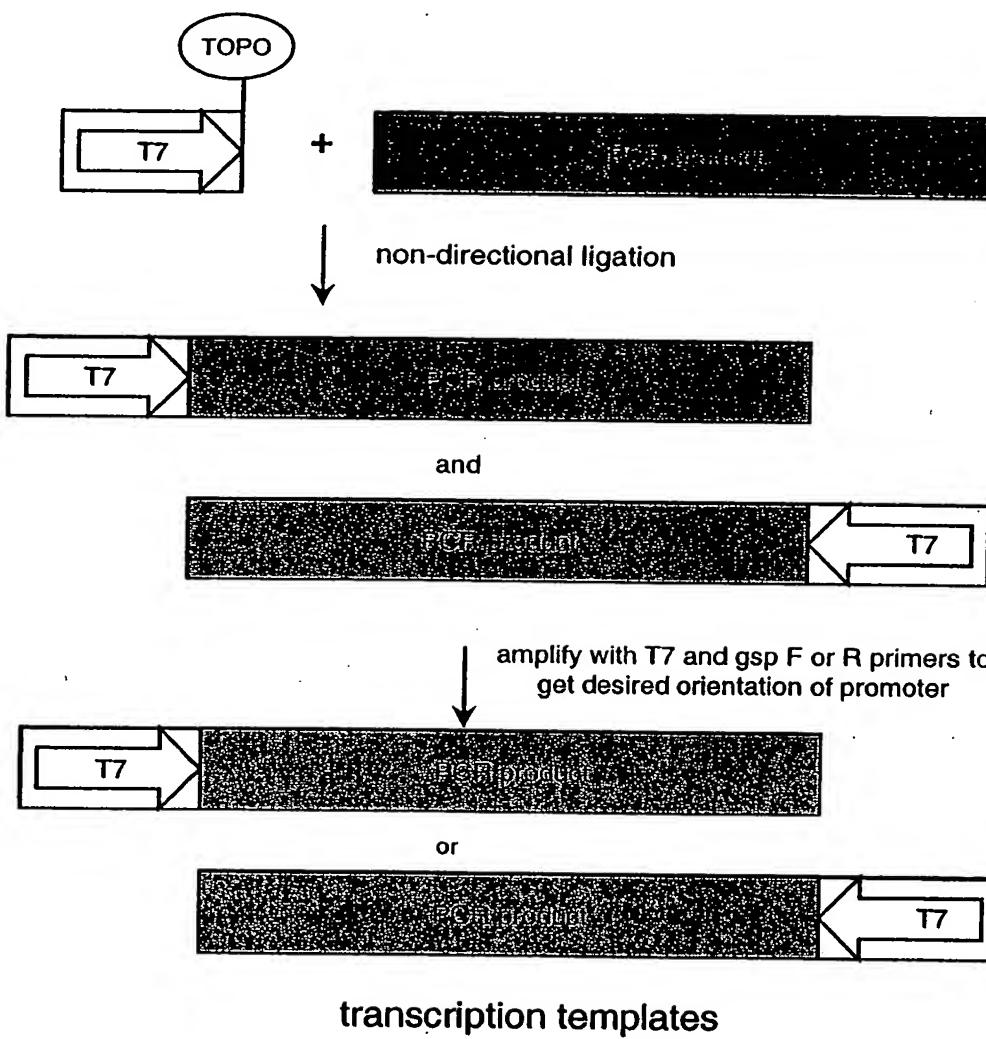
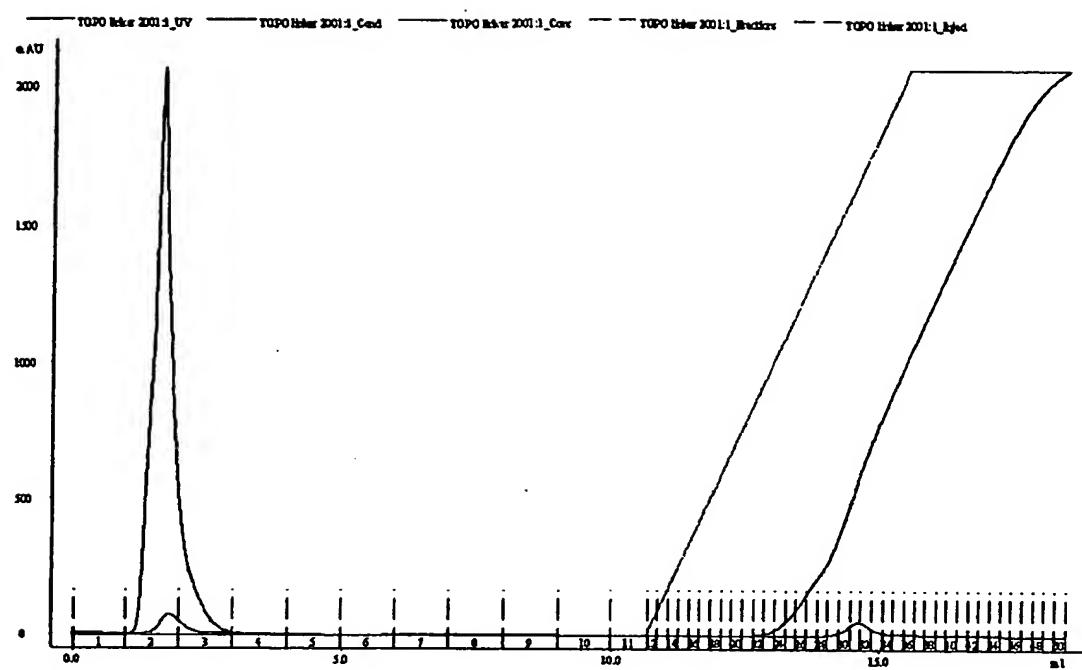
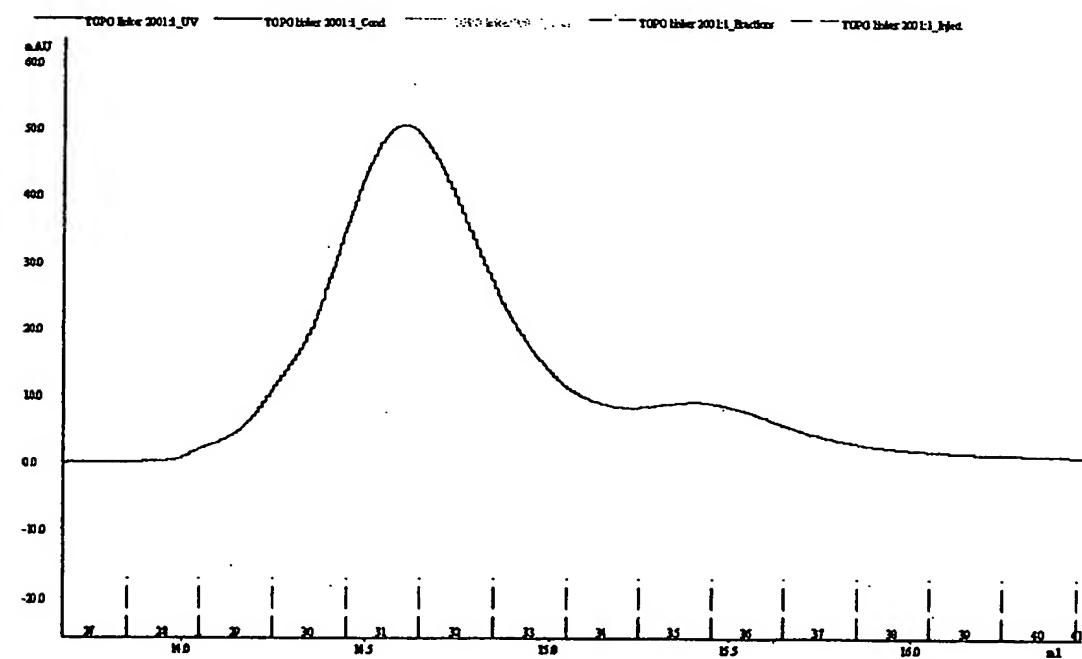
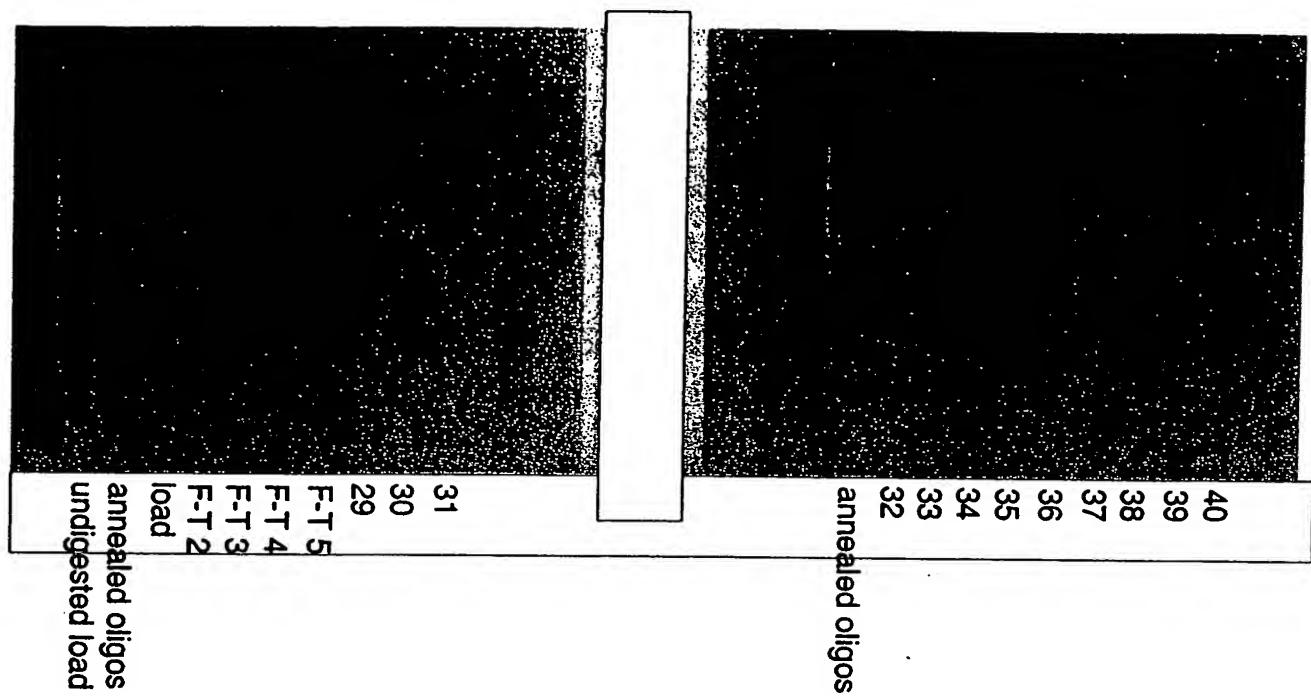


FIG. 42

A.**B.****FIG. 43**

A.



B.

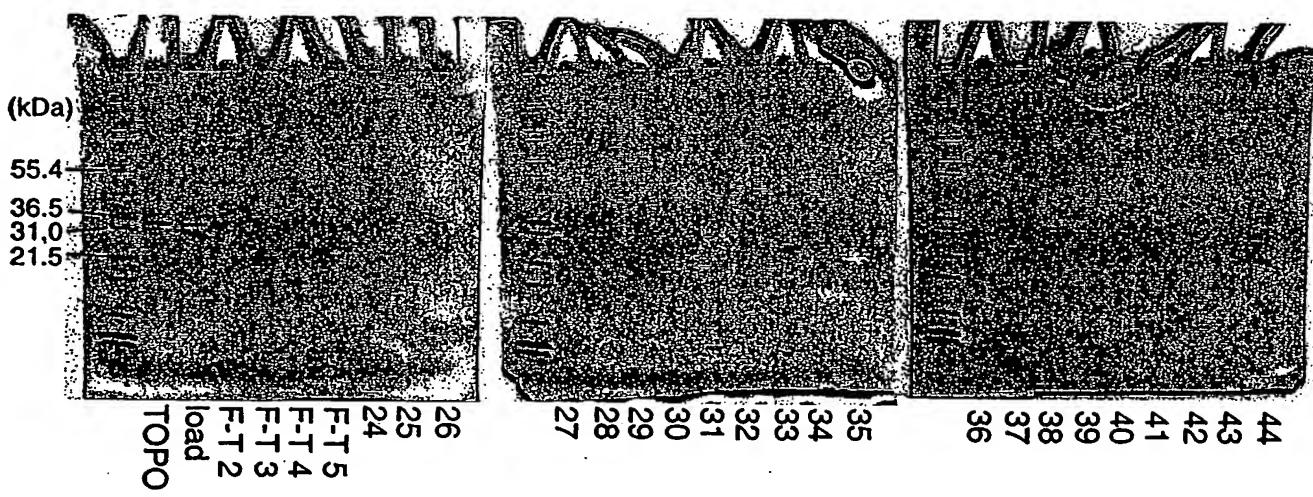


FIG. 44

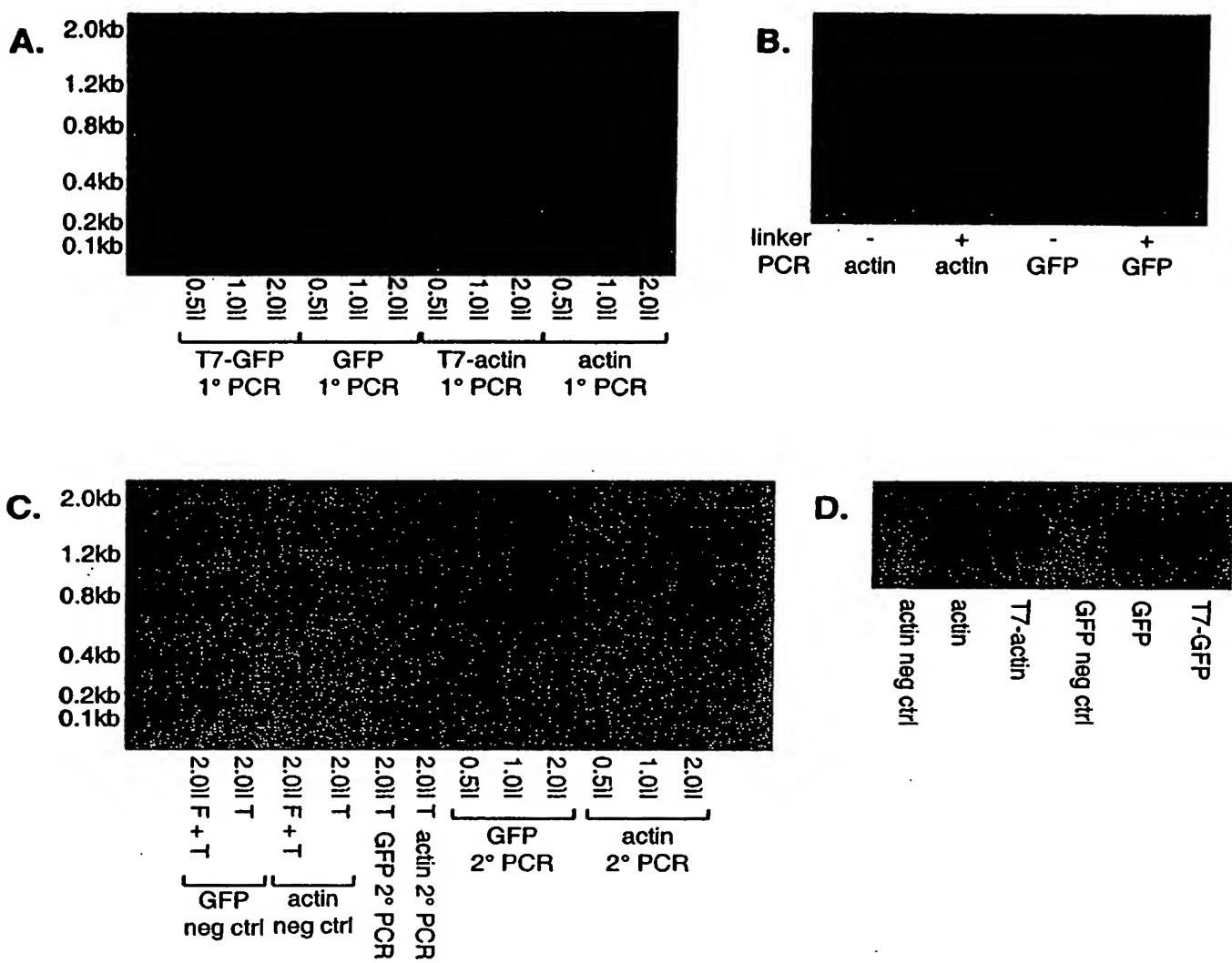


FIG. 45

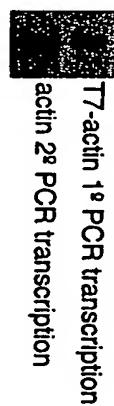
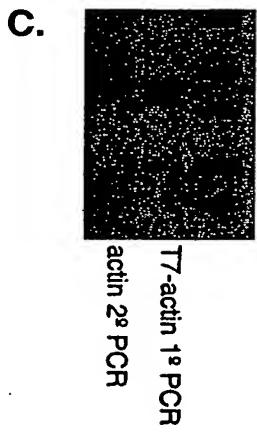
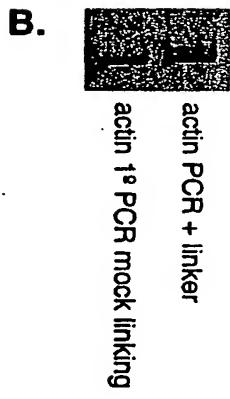
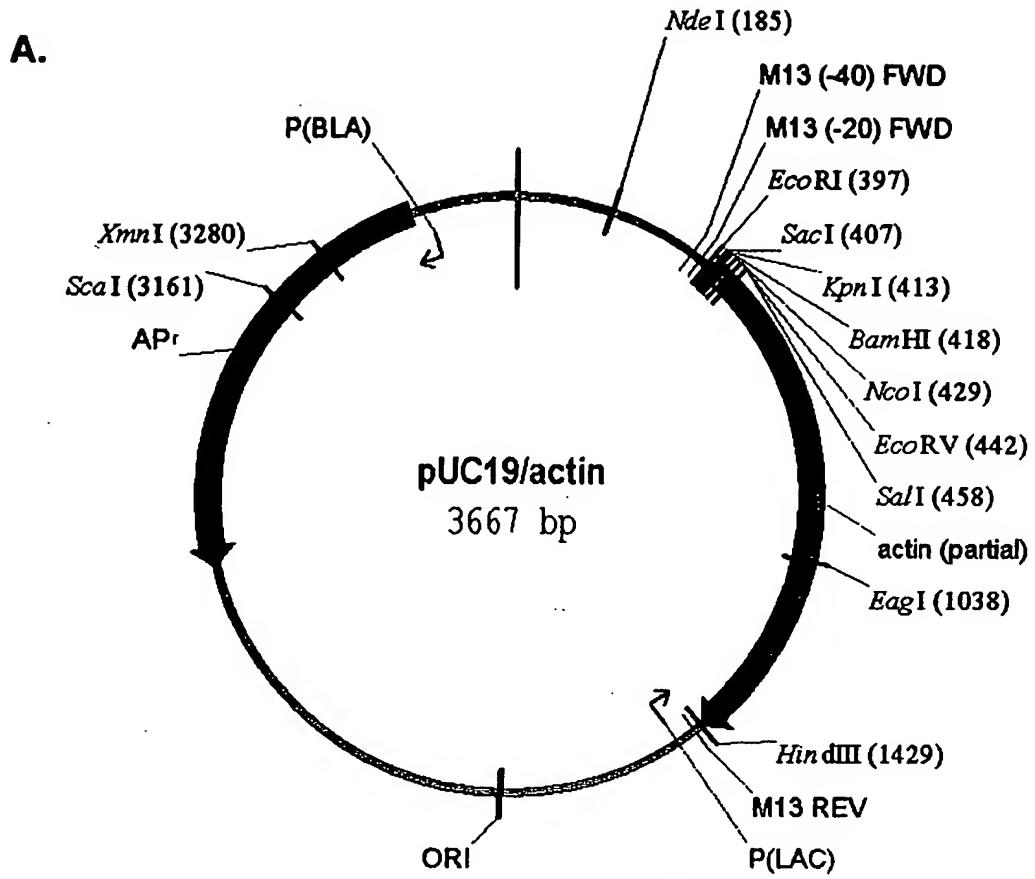


FIG. 46